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THE ROLE OF SOCIALITY AND DISTURBANCE IN SHAPING ELK (CERVUS CANADENSIS) POPULATION STRUCTURE

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in the College of Arts and Sciences at the
University of Kentucky

By

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ABSTRACT OF DISSERTATION

THE ROLE OF SOCIALITY AND DISTURBANCE IN SHAPING ELK (CERVUS CANADENSIS) POPULATION STRUCTURE

Investigating how and why individuals interact is an important component to understanding species ecology. The type and patterning of relationships (social structure) provides pertinent insight into how ecological factors such as spatial heterogeneity of resources and predation influence associations between individuals. Many taxa exhibit temporally fluid association patterns, where individuals associate with a variety of others at different rates. Ungulate species exhibit prime examples of highly fluid grouping patterns and individuals form both temporary and long-term associations. The effects of human disturbance on ungulate behavior are well documented and these changes are further exacerbated during the hunting season. Species such as elk (*Cervus canadensis*) are highly managed having subsistence, recreational, and economic value. The demographic effects of selective take or harvest regimes on population dynamics are known, but how human disturbance, including hunting, influences ungulate social structure on a fine-scale has not been explored. I aimed to investigate the relationship between human disturbance and social structure in a population of elk residing in southeastern, Kentucky, USA. I choose to focus on female elk given the importance of adult female survival to population dynamics and previous knowledge of some social affinity between females. I begin by discussing factors that influence ungulate sociality, how human disturbance can influence sociality and how a better understanding of association patterns could aid in management decisions. I then present two distinct yet vital studies to understanding this relationship: (1) investigation of survival of elk in Kentucky and (2) investigation of association patterns in a human dominated landscape. Hunter harvest is the primary cause of elk mortality in both eastern and western populations in North America and 85.2% of all elk mortalities in Kentucky were hunter harvest related. Older (> 5) males and younger (< 2) females had significantly higher hazards of dying relative to other age classes. Moreover, the establishment of a limited entry hunting area to prevent local overharvest of males had no effect on male survival, but instead may have resulted in local overharvest of females at one site residing on publicly accessible land. Female elk exhibit both weak and strong association patterns. I found that relatedness was significantly greater within sites, similar to patterns found in other cervid species. Association patterns within sites were not explained by age class; and relatedness was only positively correlated at one site. The sites investigated differed in the type and frequency of human disturbance, specifically hunting,

suggesting that the disparity in association patterns were driven by these differences. I conclude with two smaller studies, suggesting an indirect consequence of coal surface mining disturbance on ungulate foraging behavior and the potential for interstate transfer of ecto-parasites during reintroduction efforts. This research reinforces previous findings and further refines our understanding of ungulate social structure. Consideration of temporal variation in association patterns of ungulates and other species is important to quantify the effect of disturbance on population and social processes, but also to increase our understanding of dynamic structures. Quantifying the resiliency of structure to disturbance is a priority to further our understanding of the ecology and conservation of these species.

Keywords: social structure, Cervus canadensis, human disturbance, Kentucky

Brittany L. Slabach

08/02/2018

Date

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To Gramps. For seeing in me what I did not know was possible and whose constant answer of ‘Why not?’ kept me looking for more. Love Always, Long Tall

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(Fritz Slabach) who forces me to take vacations at the most inopportune times, but continuously reminds me of why I fell in love with ethology; and that getting up at 3 am is the most honored experience when you get to be a part of the golden hour. And to Mr. Michael Thamann. My confidante, my sounding board, my partner in all things. He never hesitated to assist with coding problems, brainstorm ideas until late hours in the night, or hike endless hours on nothing but a cliff bar. His love and support have been pivotal to the accomplishment of this project and I will be forever thankful for his patience, support and encouragement. To my students, whose questions in the classroom, field and hallways have continued to challenge me to be a better teacher, researcher and mentor. Keep thinking, asking questions and exploring. And to all those fellow first-generation students who don't see the possibility in themselves, keep asking yourself 'Why not?' because you can.

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CHAPTER ONE:

SOCIALITY IN A HUMAN DOMINATED LANDSCAPE

Introduction

Sociality (e.g., grouping) is expected to evolve when the net benefits of associating with conspecifics exceeds the costs (Krause and Ruxton 2002). These benefits and costs vary across time and with habitat type, predator density, and physiological state of individuals (Conradt and Roper 2003; Fortin et al. 2009). Therefore, temporally variable association patterns – where individuals associate with a variety of others at different rates – are common in many species (Krause and Ruxton 2002; Silk et al. 2014). For instance, group size of African apes varies in response to the spatial distribution and density of fruiting trees (Chapman and Chapman 2000), size of hunting packs of social canids are positively correlated with prey weight (Smith et al. 2008), and size and demographics of ungulate groups vary with predation pressure (Manor and Saltz 2003; Sundaresan et al. 2007).

Sociality is hypothesized to have evolved, in part, as an antipredator strategy (Alexander 1974; Geist 1974; Brashares et al. 2000; Kappeler and van Schaik 2002). Individuals reduce their risk of predation by being in a group (Hamilton 1971; Alexander 1974) benefiting from collective vigilance or cooperative defense (Pulliam 1973; Krause and Godin 1995). Risk of predation is temporally and spatially variable, and individuals perceive risk in their environment and adjust their behavior to avoid said risk (Kie 1999; Laundre et al. 2001). For example, if risk is predictable in both time and space individuals will avoid habitats high in risk when predators are present but utilize them when absent (Creel et al. 2005; Kohl et al. 2018).

Research has collectively shown that animals perceive humans as a predation threat (Stankowich 2008; Martin et al. 2014; Hertel et al. 2016) and wildlife globally is altering their diel patterns to avoid human disturbance (Gaynor et al. 2018). Human disturbance is both highly predictable and unpredictable. Hunting is commonly localized, occurs on fixed days and at predictable times such as weekends, dawn and dusk (Proffitt et al. 2009). Individuals adjust their behavior by avoiding areas with high road densities and human presence or reducing their use of these areas during hunting seasons (Proffitt et al. 2009; Hertel et al. 2016). In contrast, non-consumptive recreational use of landscapes can be highly unpredictable, vary in time and space, and the perceived level of risk. Therefore, mixed use landscapes create mosaics of risk that can result in conflicting selective pressures.

Here I discuss how human disturbance can directly and indirectly alter social patterns of ungulate species. First, I briefly review the evolutionary and ecological factors that influence ungulate grouping. I then discuss how human disturbance, both non-consumptive and consumptive recreation, influences sociality. The effects of disturbance are compounded across scales of structure directly and indirectly influencing association patterns, groups, and subpopulations. I then conclude by discussing how sociality and social structure has implications for management, particularly of populations residing on mixed use landscapes.

Factors Influencing Sociality

Ungulates exhibit grouping patterns ranging from pairs to large aggregations – random assortment of individuals that exhibit no organized pattern – that vary across time.

Adaptations to different habitats and predator avoidance shape how individuals are dispersed across the landscape (Geist 1974; Jarman 1974; Brashares et al. 2000). In general, larger bodied species (> 20 kg) tend to form larger groups and forage in open landscapes; whereas nutritional constraints of specialist herbivores favor smaller groups compared to their generalist counterparts (Brashares et al. 2000). Valiex et al. (2009) found that predation risk had a stronger effect on the distribution of browsing species compared to grazing species in African ungulates; and Thaker et al. (2011) reported that prey species avoidance of spatial overlap with predators was strongly influenced by body size. Within species differences also fit this trend, such as the case between cape buffalo (*Syncerus caffer caffer*) that dwell in open habitats and form groups with hundreds of individuals compared to its forest dwelling sister species (*Syncerus caffer nanus*) that form groups of less than fifty (Korte 2008).

Predation risk is a strong driver of sociality in ungulate species (Geist 1974, 2006; Jarman 1974). Individuals benefit from collective vigilance in detecting predators (Pulliam 1973; Roberts 1996), and confusing predators upon fleeing (Krause and Godin 1995). Grouping acts as a form of cover seeking behavior and the distribution of group sizes should represent those that minimize predation risk (Hamilton 1971; Alexander 1974). Species that typically reside in closed canopies and are solitary, such as white-tailed deer (*Odocoileus virginianus*; Aycrigg and Porter 1997) and moose (*Alces alces*; Molvar and Bowyer 1994) aggregate when they move into open landscapes where predation risk is higher. The probability of predation decreased by 4 - 15% with increasing group size in impala (*Aepyceros melampus*; Creel and Creel 2002) and mule deer (*Odocoileus hemionus*; Lingle 2001). Larger groups are more often detected and attacked by predators

(Hebblewhite and Pletscher 2002) and smaller less conspicuous groups form given individual risk decreases with the addition of only a few members. For example, female and male elk (*Cervus canadensis*) group size converged to 6-9 individuals in the presence of wolves in Yellowstone National Park; a smaller group size than typically observed for females and a larger group size than typically observed for males (Winnie, Jr. and Creel 2007).

Differences in life history strategies between the sexes further influence individual responses to predation and the propensity to group. Males maximize their reproductive fitness by increasing mating opportunities and often choose food over security (Ruckstuhl and Neuhaus 2000). Male elk form small all-male groups or remain solitary during winter seasons when they are highly susceptible to predators (Creel and Winnie 2005; Winnie Jr. and Creel 2007). They also show a muted response to wolf presence post rut even though they are six times more likely to be predated upon compared to females (Mech et al. 2001; Creel and Winnie 2005), suggesting that nutritional constraints are stronger determinants of individual behavior post rut.

Alternatively, females select security over high quality resources and remain in groups year-round. Thus, increasing the potential for relationships between individuals and stable associations between females have been documented in both cervid and bovid species (Hirotani 1990; Vervaecke et al. 2005). Early studies of elk social structure documented stable female subgroups that maintained spatial proximity to each other and became small groups when larger groups fragmented (Altmann 1956). Other studies have documented social units of female elk and red deer (*Cervus elaphus*) via home-range overlap or co-herd membership (Clutton-Brock et al. 1982; Jenkins and Starkey 1982; Millspaugh et al. 2004).

However, these female association patterns have not been reported in all populations (Knight 1970; Houston 1982). Females exhibit strong antipredator responses including decreased group size, increased vigilance behavior and the use of alternative habitats (Hunter and Skinner 1998; Winnie, Jr. and Creel 2007; Clutton-Brock 2009). These behavioral changes (e.g., risk effects) directly influence sociality and changes in grouping patterns may be predictable based on individual association preferences. Risk effects can also result in other physiological effects such as decreased body condition (Creel and Christianson 2008; Creel et al. 2011), although data is conflicting (Middleton et al. 2013).

Human Disturbance and Sociality

Human recreational use of landscapes including all-terrain vehicles, hikers, and road vehicles influence ungulate behavior (Wisdom et al. 2005; Ciuti et al. 2012b). These behavioral responses are synonymous to those elicited by non-human predators and they are fairly ubiquitous across species (Stankowich 2008). The strength of individual responses varies with disturbance type, unpredictability and therefore, perceived risk. For example, big horn sheep (*Ovis canadensis*) elicited a stronger behavioral response to hikers compared to other forms of disturbance due to the spatial unpredictability of hikers and their propensity to approach sheep (Papouchis et al. 2001). In populations not subjected to hunting individuals perceive areas of human use as low risk and instead use these areas as refuge from non-human predators (Berger 2007; Shannon et al. 2014). Temporally fluid association patterns are hypothesized to be the result of individuals balancing ephemeral changes in risk with energetic needs (Gower et al. 2009). Therefore, group size and membership may be more variable in populations residing on mixed use landscapes given

individuals are responding to various types of perceived risk. The creation of riskier landscapes has also been suggested as a management tool to reduce the frequency of human-wildlife conflicts by altering the spatial distribution of individuals (Cromsigt et al. 2013). Increased variability and fragmentation of groups can result in decreased recreational viewing opportunities outside of the hunting season and could augment disease transmission if individuals are associating more with others. Yet it could also prevent further disease spread if groups remain fragmented. Smaller groups are also more vulnerable to predation and negative stochastic effects, particularly in spatially segregated populations (Mooring et al. 2004; Festa-bianchet et al. 2006). Therefore, more specific information of how human disturbance influences association patterns are needed.

The primary source of adult mortality in most ungulate populations is hunter harvest (Festa-Bianchet 2007; Milner et al. 2007). And ungulate response to human disturbance is most pronounced during the hunting season when individuals seek refuge in habitats with lower human and road densities (Lyon and Christensen 2001; McCorquodale 2013; Profitt et al. 2013; Thurfjell et al. 2017). These responses vary by habitat and level of risk, but responses are typically stronger in open habitats with higher perceived risk. Elk, impala and bison (*Bos bison*) all form smaller groups in the presence of hunters (Manor and Saltz 2003; Fortin et al. 2009; Proffitt et al. 2009), suggesting individuals are selecting an antipredator strategy of smaller more conspicuous groups. Changes in group size can decrease hunting opportunities and result in the disproportionate harvest of groups that reside in more accessible areas (Proffitt et al. 2013). For example, differences in public access in Kentucky resulted the local overharvest of females at one site (Chapter 2).

Reducing the local density of females within an area could positively influence the availability of economically valuable males. Male ungulates are more strongly affected by density dependent factors (Clutton-Brock and Coulson 2002). And a reduction in female density of red deer and mule deer (*Odocoileus hemionus*) groups increased the resident male population leading to increased male harvest yields (McCullough 2001; Clutton-Brock et al. 2002). This observed effect was greatest when neighboring areas/management units maintained higher female densities, thus reducing male immigration (Clutton-Brock et al. 2002). Therefore, changes in group size and spatial distribution could positively affect management objectives, but these effects must be considered in relation to sex-specific densities, hunter access and the densities of individuals on surrounding sites.

The targeted removal of individuals directly and indirectly select for particular phenotypic characteristics having consequences for social processes and observed group and subpopulation level effects. Removal of dominant males negatively affects fecundity in species with dominance hierarchies or harem structures (Milner et al. 2007). Hunter preference for large antlers or horns can skew male age structure decreasing fecundity, recruitment (Milner et al. 2007) and the effective breeding population size (Allendorf and Hard 2009). The long-term consequences of artificial selection on male phenotypic characteristics has remained a concern, although the true genetic implications of any preference are unclear (Allendorf and Hard 2009; Heffelfinger 2018). One of the more well-known examples from Coltmann et al. (2003) showed that hunter preference for male horn size in bighorn sheep resulted in a decline in male body mass and horn size. Yet more recently Coulson et al. (2018) demonstrated that the rate of change reported by Coltmann

et al (2003), and others in the same species, was magnitudes faster than could occur under standard genetic assumptions.

Hunting can also select for particular behaviors. For instance, bold individuals, of either sex, that have higher rates of movement and use open areas more frequently are disproportionately killed by hunters in elk and red deer (*Cervus elaphus*) populations (Ciuti et al. 2012a; Lone et al. 2015). This could result in selective pressure that is antagonistic to strategies of natural predator avoidance (e.g., selecting for a hiding strategy versus a running strategy) and increase the risk of individual mortality in multiple predator landscapes (Eberhardt and Pitcher 1992; Wright et al. 2006). This effect could be further compounded in populations subjected to different types of recreational use. In addition, selection for bold individuals could reduce connectivity between subpopulations having both positive and negative effects on population viability and processes such as disease or information transfer.

Learned behaviors such as migration routes (Jesmer et al. 2018), avoidance of hunters (Thurfjell et al. 2017) and location of resources are important for individual survival and population persistence. Hunters disproportionately harvest reproductive females compared to non-human predators (Eberhardt et al. 2007; Milner et al. 2007). For example, the average age of a hunter killed female was 5 (B.L. Slabach unpublished) and 5.7 (J. Banfield, Pennsylvania Game Commission, pers. comm.), respectively. Increased removal of adult females could have long-term consequences on population viability through the loss of social information. Our knowledge of these phenomena in ungulates is limited, although effects have been observed in other species. For instance, when a matriarch is removed from an elephant herd the remaining members were unable to distinguish social

cues between dominant and unaffiliated individuals; an affect observed decades post culling (Shannon et al. 2013). Removal of adult females could also affect group stability, particularly in species that form stable associations, increasing the potential for fragmentation. Younger age class individuals are more prone to exploratory behavior (Clutton-Brock et al. 1982; Haydon et al. 2008). Although this exploratory behavior could play a pivotal role in maintaining connectivity between groups or subpopulations (Williams and Lusseau 2006; Wiszniewski et al. 2010), it can also increase the propensity for groups composed of predominantly younger age classes to fragment (Dolev et al. 2002; Ciuti et al. 2012a).

The effects of human disturbance on populations are multifaceted. A general pattern of these effects, particularly on population level processes, is still unclear due to how factors are compounded across social scales (e.g., individuals, groups, subpopulations; Tablado and Jenni 2017). There has been a renewed interest in describing the social structure of species (Archie et al. 2006; Aureli et al. 2008; Smith et al. 2008; Carter et al. 2013). The integration of social network theory into studies of behavior have provided an analytical framework and set of statistical tools to investigate the type and patterning of individual relationships and the emergent structures that form from them (Whitehead 2008; Pinter-Wollman et al. 2014). Thus, providing a framework to investigate mechanisms that influence association patterns within and across social scales. Quantifying associations has implications for studying information transfer (Krutzen et al. 2005), predator-prey relationships (Krause and Ruxton 2002; Fryxell et al. 2007), and disease dynamics (Cross et al. 2004; Vander Wal et al. 2012b), all of which have application to the conservation and management of species.

Sociality and Management

Dynamics of large ungulates are affected by the sex and age structure of populations (Gaillard et al. 1998, 2000; Clutton-Brock and Coulson 2002). Management strategies focus on either controlling the adult female cohort or improving survival rates and optimizing hunter opportunity of valuable trophy males (Lyon and Christensen 2002). The total population size is regulated via changes in population demographics and these changes occur on a local or regional scale. Management efforts are either directed towards the whole population or management units that are geographically defined boundaries commonly created using information of local density and the availability of land for hunters to access. Therefore, management units represent subpopulations of various sizes that may be spatially fragmented or may frequently interact.

We use changes in behavior and spatial distribution on a local scale to inform management decisions, decreasing the probability of overharvest or increasing the number of available males (Bender and Miller 1999). And we make these decisions without a thorough understanding of how temporal changes and dynamism in associations influence group and subpopulation structure. It is difficult to tease apart the effects of disturbance on social processes because we are managing for changes at a broad scale without necessarily considering the fine scale structure that drives the observed patterns on the landscape. Moreover, the effects of additive mortality via hunting of the female cohort may remain masked in populations that are held below ecological carrying capacity (Bowyer et al 2014). Therefore, investigating structure and the temporal changes in associations, particularly of females, at the individual and group level would aid our understanding of social processes and better inform management efforts.

Reintroduction of elk back into the eastern portion of the species range in North America has provided a unique opportunity to investigate the relationship between human disturbance and social structure. Reintroduction efforts have been occurring since the early twentieth century and currently 12 populations of elk have either been established or recently introduced. Most eastern populations are small, below 1000 individuals, although Michigan (~ 1371; MIDNR Strategic Plan 2015) and Kentucky (~10,000; KDFWR 2015) boast higher current population estimates. These populations experience less predation by non-human predators compared to their western counterparts, yet mortality due to factors such as vehicle collisions or nuisance culling are more frequent (Keller et al. 2015). The eastern United States has a high percentage of wildland-urban interface where urban structures are common in forests and grassland areas (Radeloff et al. 2005); and much of the eastern region is within 1 km of the nearest road (Riitters and Wickham 2003). Thus, eastern populations of elk are more exposed to high road densities, humans and human disturbance compared to their western counterparts providing an opportunity to investigate the relationship between disturbance, structure, and demographics at local, regional and population scales.

Quantifying structure in wild populations is logistically difficult. The fluid social structure of ungulates necessitates a large number of marked individuals, and habitat and terrain can limit observation capability. Many eastern populations have a high percentage of marked individuals and in some cases, the entirety of the population is marked including neonates. Habitat use and resource selection, population demographics and vital rates including survival, cause-specific mortality and fecundity are all monitored as part of reintroduction or management efforts. And social structure has been anecdotally

considered in many of these populations with the identification of a herd or herds defined as individuals who remain in an area throughout an annual cycle; satellite herds defined as individuals that at least spatially overlap with others during part of an annual cycle; or in some cases core groups and groups. Even my colleagues and I (Chapter 2) use the term herd to define a site; which clearly defines the area which females remain for the annual cycle. But represents only the site of capture for males. Clarifying these subtle inconsistencies in definitions would provide a unified framework to incorporate investigation of social structure into already existing research. Therefore generating a distribution of structural differences and behavioral responses from across a variety of landscapes and human disturbance types.

Ungulates respond to predation risk in a variety of ways and monitoring group level changes is only one component to a more complex socio-ecological process. Hunting creates a strong temporal pulse in predation risk and the resulting behavioral changes can make it difficult to meet management objectives. Harvest of individuals can directly and indirectly influence social processes that further compound the effects of predation risk on longer temporal scales. Moreover, non-consumptive recreational landscape use creates a mosaic of risk that can result in conflicting selective pressures and further influence spatial distribution of individuals both outside and during the hunting season. Incorporating studies of social structure into applied wildlife management will increase our understanding of the social and ecological factors that influence structure but will also aid in our capabilities to manage populations for long-term, sustainable use in mixed landscapes. Variation in structure is an important factor in determining population persistence in changing environments and furthering our understanding of the social

processes that underlie the temporally fluid associations common across taxa would further our ability to predict how populations will respond to environmental changes.

CHAPTER TWO:

SURVIVAL AND CAUSE-SPECIFIC MORTALITY OF ELK (CERVUS CANADENSIS) IN KENTUCKY

Coauthors: J.T.Hast, S.M.Murphy, W.E.Bowling, R.D.Crank, G. Jenkins, K.L. Johannsen, and J.J.Cox – A version of this chapter is currently under review for publication in the *Journal of Wildlife Biology*

Introduction

Ungulate population management is largely focused on maintaining populations that satisfy various, often competing stakeholder groups including both hunters and non-consumptive users. In North America, legal hunter harvest and predator control remain the primary management tools used to manipulate ungulate population dynamics (Stalling et al. 2002). Population-specific management strategies commonly focus on either preserving adequate security habitat for economically valuable trophy males in order to improve survival rates and optimize hunter opportunity (Lyon and Christensen 2002); or control the adult female cohort given that the survival and fecundity of adult females strongly dictates ungulate population growth (Gaillard et al. 1998, 2000). Ungulate avoidance of disturbance is well documented and most pronounced during the hunting season (Stankowich 2008; McCorquodale 2013; Proffitt et al. 2013; Thurfjell et al. 2017). Hunting season structure, length and hunter density can result in behavioral shifts by ungulates (e.g., increased vigilance and movement patterns) that can decrease hunter success or cause disproportionate harvest of population cohorts, making it difficult to meet management objectives (Stalling et al. 2002; Proffitt et al. 2013).

North American elk (*Cervus canadensis*) were overexploited and eventually extirpated from their native range in the eastern USA by the late nineteenth century (Larkin et al.

2001). Reintroductions during the 1910s created a few isolated elk populations in Pennsylvania and Michigan (Larkin et al. 2001), but the species remained absent from the majority of its historical eastern range through most of the twentieth century. Between 1997 and 2002, a total of 1,541 elk were reintroduced to the eastern portion of Kentucky, USA, to establish a resident population (Larkin et al. 2001; Kentucky Department of Fish and Wildlife Resources [KDFWR] 2015). Population growth was rapid, reaching a presumed abundance of 10,000 to 12,000 elk by the 2010s (KDFWR 2015). The first elk hunting season in Kentucky occurred during 2001, and hunting has subsequently remained the only applied management strategy since large carnivores are absent from the elk restoration area (KDFWR 2015); although, coyotes (*Canis latrans*) are present and American black bears (*Ursus americanus*) have recently expanded range towards core elk population areas (Larkin et al. 2002a; Murphy et al. 2016). Approximately 700 – 1,000 elk hunting permits have been available annually over the past decade.

Research on elk in Kentucky has focused on survival, movement patterns and female elk pregnancy rates, all of which are important for population establishment and subsequent growth (Larkin et al. 2001; Larkin et al. 2002a,b; 2003b). Only six males were part of initial research, and males have remained largely unstudied in this population. Following anecdotal reports from hunters and guides that the number of male elk had declined by the early 2010s, KDFWR implemented antler point restrictions and spike-only harvest permits to attempt to reduce harvest of male elk (KDFWR 2015). However, because the majority of lands in the Kentucky elk restoration area are privately owned, only 13.0% of the 16,802 km^2 area is publicly accessible (KDFWR 2015), there is an increased chance for hunters to overexploit elk on public lands. Consequently, state wildlife managers implemented a zone

system in 2008 to attempt to spatially disperse hunters across the landscape and prevent hunters from clustering on particular lands. With continued concern over the number and availability of male elk to hunters, limited-entry areas (LEA) were established within zones in 2013. A LEA system is designed to limit the number of permits and/or hunters allowed within a specified area and has been shown to positively influence the number of male elk post implementation, particularly of mature age classes (Bender and Miller 1999). KDFWR aimed to minimize the likelihood of local overharvest by forming LEAs that encompassed areas with a high density of public land (KDFWR 2015).

Given the dated information on females and complete lack of information on males, my colleagues and I sought to (1) estimate survival and cause-specific mortality probabilities for both male and female elk, and (2) investigate if the implementation of the limited-entry area harvest strategy influenced elk survival. We hypothesized that hunter harvest would be the primary cause of mortality for both sexes in this population due to the relative lack of non-human predators within the area. Specifically, we hypothesized that the change to a limited-entry area system would increase male elk survival rates, as has been previously reported elsewhere. Finally, we hypothesized that survival rates for individual elk herds within the limited-entry area would vary based on the ownership type of lands that those herds primarily occupied.

Study Area

Our study area was approximately 300 km^2 (1.8% of the elk restoration area) and was located in the Cumberland Plateau physiographic region of southeastern Kentucky, USA (Table 2.1). This area is characterized by rugged topography, including mountains and

ridges of 300-1300 m in elevation, with deep drainages and narrow valleys. The climate is considered temperate humid continental (Hill 1976; Overstreet 1984), with an average annual temperature of 13° C, mean winter temperature of 4° C, and mean summer temperature of 22° C. Coal extraction in the form of mountain top removal surface mining was the dominant land use and has dramatically altered the topography and biota of the landscape, resulting in a mosaic of open grasslands, second and third growth forests, and active and repurposed inactive surface mines (Larkin et al. 2001; Pericack et al. 2018). On repurposed inactive mine sites, dense forest and steep mountains are replaced with man-made contoured valleys and mesas that were replanted with grasses and low-shrub vegetation following mining (Pericack et al. 2018). Both active and repurposed surface mines exist within our study area, including a ~9 km^2 tract of repurposed surface mine that was transitioned to a KDFWR Wildlife Management Area (PVB). Additional sites of interest included a public hunting area that was 32 km^2, comprised of planted grasslands and forested areas with all-terrain vehicle and horse trails (ATV); and a 59 km^2 second growth forest that is a University of Kentucky-owned research forest where hunting was not permitted.

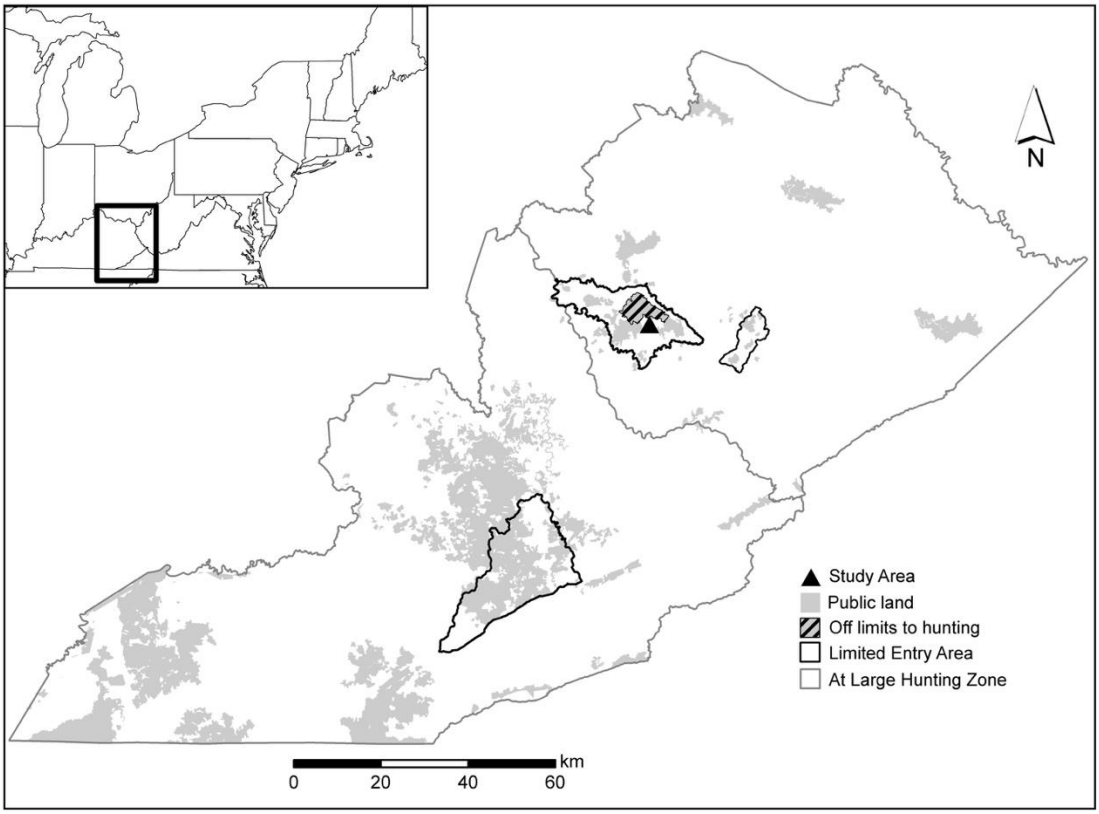

Figure 2.1. Map of the elk restoration area and elk hunting zones in southeastern Kentucky, USA. The elk restoration area is comprised of a 16,802 km^2 area that encompasses the southeastern portion of the state (inlay). Elk hunting zones within the restoration area changed during the course of our study. The area was divided into six hunting zones for the 2011 and 2012 seasons with limited publicly accessible lands. A limited-entry system was established in 2013 that include two 'at large' areas (outlined in gray) and three limited-entry areas (outlined in black). Our study area was completely encompassed within one of these limited-entry areas.

Methods

Capture and Monitoring

We used corral trapping to capture female elk and juvenile (1.5 yoa) males, whereas we used free-range darting to capture subadult and adult males and adult females. We captured elk after each annual elk hunting season ended; male elk captures occurred from 22 January to 31 July for each of three years (2011, 2012, and 2013). Female elk captures occurred from 1 February to 31 March for each of two years (2013 and 2014). We ceased capture by April 1 each year to reduce the risk of injury to females and unborn calves. We chemically immobilized elk using Carfentanil citrate (Zoopharm, Windsor, Colorado, USA) at a dosage of 0.005–0.020 mg/kg of estimated body weight (Kreeger and Arnemo 2007), administered via a rifle-propelled dart or jabstick. We approached immobilized elk within five minutes of administering anesthetic and placed all elk in sternal recumbency to reduce the potential for bloat and aspiration. When possible, we did not chemically immobilize calves or yearling females that were captured in corral traps; instead, we used a working chute with a cattle head gate (Tarter Gate Cattlemaster Series 3, Dunnville, KY, USA) to secure the animal. We blindfolded all captured elk to reduce visual stressors and applied ophthalmic ointment to the eyes to reduce corneal damage. Respiration, pulse, rectal temperature, and mucous membrane color were checked every five minutes during immobilization.

We outfitted each captured elk with either a very high frequency (VHF) radio collar (model: LMRT-4; Lotek, Newmarket, ON) that was individually marked using a colored banding pattern or a global positioning system (GPS) collar (model: 8000 MGU; Lotek, Newmarket, ON); GPS collars were only deployed on males. We also applied numbered

cattle ear tags to assist with visual individual identification. For all adult elk (> 2 yoa), we administered a local injection of 1mL of lidocaine at a dosage of 20 mg/kg to the mental foramen prior to extracting one lower incisor (I4) for age determination via cementum annuli analysis (Fancy 1980). Yearling elk were aged by the presence of one pair of permanent incisors (Hudson and Haigh 2002). We collected 20 mL of blood from the jugular vein of each elk for blood parameter analysis. We recorded total elk body length, hind foot length and chest girth for all captured elk, as well as the number of antler points, main beam lengths, length of inside spread, beam circumference and sword point length for all male elk. Antler measurements were taken for male elk in velvet (n = 39) but were considered incomplete. Any signs of previous injuries or capture injuries were also noted. We used the antagonist Naltrexone hydrochloride at a dosage of 100 mg per/1 mg of Carfentanil citrate administered to recover immobilized elk, which we delivered via an intramuscular injection into the shoulder or hip. We then monitored elk until they became ambulatory and were out of immediate danger or self-injury (~ 4.5 min on average). All capture and immobilization procedures were approved by a University of Kentucky International Animal Care and Use Committee (protocol #2010-0726).

The Kentucky elk hunting season began in mid-September and continued for approximately 120 days annually during our study. The season was partitioned into five segments that included both separate and combined antler and antlerless archery and two weeks of modern firearm for each class. Archery hunting closed during the four weeks (cumulative) of firearm hunting. We conducted mortality monitoring weekly or bi-weekly via ground or fixed wing air telemetry from mid-February to 31 July. We increased mortality monitoring to 3 times per week from August 1 to mid-February, encompassing

one month prior to the hunting season through one month following the hunting season. We monitored males fitted with GPS collars twice per week via remote downloading of their activity and location data. We investigated all mortality signals within ≤ 12 hours of detection. We submitted dead elk either to the Lexington Diagnostic Disease Center (University of Kentucky, Lexington, KY) or performed a field necropsy at the site of mortality. If viable, we accessed the brain via a lateral section through the skull, and both hemispheres were then formalin-fixed and submitted to the Southeastern Cooperative Wildlife Disease Study (University of Georgia, Athens, GA) for meningeal worm (*Parelaphostrongylus tenuis*) confirmation testing (Bender et al. 2005).

Survival and Cause-specific Mortality

We used Cox proportional hazards regression, adjusted for staggered entry and right-censoring implemented in the R software package 'survival' (Therneau and Grambsch 2000; Therneau 2015; R Core Team 2018), to investigate the factors that may have influenced sex-specific elk survival. We evaluated the primary Cox regression assumption of proportional hazards for each variable in each fitted model by plotting the scaled Schoenfeld residuals against survival times and via a chi-square significance test implemented via the cox.zph function in the survival package (Therneau and Grambsch 2000; Therneau 2015). We computed variance inflation factors (VIF) to investigate multicollinearity between variables and we removed variables from the analysis if multicollinearity was detected (Tabachnick and Fidell 2001). We clustered elk in the analysis by individual identification number because some individuals were monitored for > 1 year and > 1 age class (i.e. correlated observations). We stratified all models by year

to produce annual survival probabilities, thereby permitting evaluation of the influence of the limited-entry area on survival. We used Akaike's Information Criterion corrected for small sample size (AIC_c) for model selection and considered all models ≤ 4 ΔAIC_c competing (Burnham et al. 2011). We obtained sex-specific estimates by producing Kaplan-Meier survival curves from fitted Cox proportional hazards models. We estimated annual survival from the 6 February to 5 of February of the following year to encompass new capture efforts and the totality of the hunting season, including any potential wounding loss mortalities for a given year. To estimate cause-specific mortality probabilities that appropriately accounted for competing risks, we used nonparametric cumulative incidence functions implemented in the R package 'cmprsk' (Heisey and Patterson 2006; Gray 2014).

We fit three *a priori* Cox proportional hazards models for each sex that included additive combinations of age class and herd location as predictor variables. We grouped males and females by age class differently to reflect the differing reproductive values (Noyes et al. 1996; Wright et al. 2006). Females were grouped as yearlings (< 2 yoa, n = 22), adult (2 – 8 yoa, n = 62), and old adult (≥ 9 yoa, n = 9), whereas males were grouped as juveniles (2 yoa, n = 31), subadults (3 yoa, n = 34), young adult (4 yoa, n = 39), adults (5 yoa, n = 28), and old adult (≥ 6 yoa, n = 19). Herd location represented the general area where each elk was captured, which differed by landownership type. We calculated antler scores for males defined as a compilation score of antler characteristics that included main beam length, antler beam circumference, total points, and brow length for each side and antler spread, but excluded these scores from our analyses given measurements were incomplete for several males. Recaptures only occurred to recover GPS collars in the event of a collar failure or if a collar prematurely dropped from an elk. Therefore, we could not

obtain updated antler measurements for males that were monitored >1year unless a mortality occurred.

Results

We captured 244 elk (151 male and 93 female) during 2011 – 2014; collars on two females and six males dropped off prematurely prior to the end of the study or death occurring, which we right-censored. Two females and one male died from capture myopathy and were omitted from the analyses. We euthanized one female and five males because of deteriorating body condition and increased behavioral abnormalities, but we retained these elk in the analyses. We removed four additional males from our data set because of incomplete records. Thus, monitoring data from a total of 91 females and 146 males (n = 237 total elk) were used in our analyses.

Among the remaining 237 elk, a total of 155 (65.4%) died during our study period. Hunter harvest was the primary cause of mortality, with 85.2% (132/155) of mortalities being from hunter harvest or wounding loss (i.e., shot by a hunter but not recovered; Table 2.1A). Of those harvest-related mortalities, 59.1% were from firearm, 41.0% were from archery (including crossbow; Table 2.1B). Wounding loss from archery was not documented in female elk nor was wounding loss from firearms documented in male elk. Nine males succumbed to death from diseases compared to just 3 females (total disease frequency = 5.1%). Diseases documented included lungworm (*Dictyocaulus viviparous; n* = 1 F), meningeal worm (*P.tenuis; n* = 1 F and 9 M), and sulfur toxicity (n = 1 F). Other non-harvest causes of mortality were only documented in male elk, with 11 males (4.6%) succumbing to causes such as poaching (n = 1), vehicle collisions (n = 4), fence

entanglement (n = 2), euthanasia after becoming trapped in a mine slurry pond (n = 1), and unknown causes (n = 3).

Table 2.1. Causes of elk mortality in southeastern Kentucky. (A.) A total of 237 elk (female = 91; male = 146) were radio-monitored from 2011 to 2015, of which 155 (65.4%) died. (B.) A total of 132 elk died due to legal harvest or wounding loss (female = 52; male = 80), and (C.) 46.3% of all female deaths due to rifle (19/41), including wounding loss occurred within one herd.

A.

	Overall	Male	Female
Legal harvest	49.8% (118)	47.9% (70)	52.7% (48)
Wounding loss	5.9% (14)	6.8% (10)	4.4% (4)
Disease	5.1% (12)	6.2% (9)	3.3% (3)
Other	4.6% (11)	7.5% (11)	0.0% (0)
Censored	3.4% (8)	4.1% (6)	2.2% (2)

B.

	Overall	Male	Female
Rifle	59.1% (78)	46.3% (37)	79.0% (41)
Archery	41.0% (54)	54.0% (43)	21.1% (11)

C.

	ATV	PVB	SF	SJ7	SC
Rifle	46.3% (19)	24,4% (5)	12.2% (10)	12.2% (5)	4.9% (2)
Archery	18.2% (2)	27.3% (2)	27.3% (3)	27.3% (3)	9.1% (1)

Survival

For male and female elk, ranges of VIF for the predictor variables were 1.03 – 1.44 and 2.12 – 5.51, respectively; therefore, we considered multicollinearity low and retained variables in our analyses. Because some support existed for male location violating the proportional hazards assumption ($p = 0.06$), although statistically insignificant at the 95% confidence level, we included an interaction between time and herd location in all models that included herd location. Only one model was supported for males (≤ 4 ΔAIC_c), which suggested that survival was influenced by age class only (Table 2.2). Estimates of male elk survival was 0.70 (95% CI = 0.58–0.86), 0.43 (95% CI = 0.34–0.55), and 0.57 (95% CI = 0.47–0.69) for 2011, 2012, and 2013, respectively (Table 2.3). Males 5 and ≥ 6 years-of-age had 1.83 (95% CI = 1.13–2.98) and 2.26 (95% CI = 1.30–3.95) higher hazard ratios, respectively (Table 2.4). Three models were supported for females (≤ 4 ΔAIC_c); the top two models both included age class and only differed by the inclusion or exclusion of herd location, whereas the third model excluded age class but included herd location (Table 2.2). Given the support for those predictor variables, we only present results from the most parsimonious top model that included both age class and herd location. Estimates of female elk survival was 0.65 (95% CI = 0.50–0.83), 0.69 (95% CI = 0.59–0.84), and 0.67 (95% CI = 0.54–0.84) for 2013, 2014, and 2015, respectively (Table 2.3). Yearling females had a 3.84 (95% CI = 1.52–9.70) higher hazard ratio, and females in the PVB and SJ7 herds had 0.39 (95% CI = 0.16–0.97) and 0.27 (95% CI = 0.09–0.83) lower hazard ratios, respectively (Table 2.4).

Table 2.2. Model selection of stratified Cox proportional hazards models explaining survival of male and female elk in southeastern Kentucky from 2011 to 2015. We stratified by year to produce annual estimates of survival for comparisons and to evaluate the efficacy of a limited-entry area that was established in 2013. We considered the influence of age class and herd location (Herd) on survival of both sexes. We also included an interaction (:) between herd location and time on male survival to overcome violation of the proportional hazards assumption for Herd. We clustered our data by elk identification number (ID) because some individuals

Model	*K*	AIC_c	ΔAIC_c	logLik
Bulls				
Age Class + Strata(Year) + Cluster(ID)	4	795.44	0.00	–393.51
Age Class + Herd + Herd:Time + Strata(Year) + Cluster(ID)	13	804.82	9.38	–387.30
Herd + Herd:Time + Strata(Year) + Cluster(ID)	9	814.00	18.56	–397.02
Cows				
Age Class + Herd + Strata(Year) + Cluster(ID)	6	416.43	0.00	–201.34
Age Class + Strata(Year) + Cluster(ID)	2	418.01	1.58	–206.89
Herd + Strata(Year) + Cluster(ID)	4	420.25	3.82	–205.71

Table 2.3. Annual survival probabilities for male and female elk in southeastern Kentucky. Survival probabilities and associated 95% confidence intervals are from the top stratified sex-specific Cox proportional hazards models.

	2011	2012	2013	2014	2015	$\bar{x}$
Males	0.70 (0.58–0.86)	0.43 (0.34–0.55)	0.57 (0.47–0.69)	–	–	0.57 (0.45–0.71)
Females	–	–	0.65 (0.50–0.83)	0.69 (0.59–0.81)	0.67 (0.54–0.84)	0.67 (0.53–0.81)

Table 2.4. Hazard ratios of variables related to time to death from the top stratified Cox proportional hazards models. One level of each variable was used as a reference and was thus part of the intercept.

Variable	β	HR (95% CI)	*Z*	*P* > |*z*|
Bulls				
Age Class				
2	–0.78	0.46 (0.18–1.17)	–1.62	0.10
3	–0.26	0.77 (0.40–1.47)	–0.79	0.43
5	0.61	1.83 (1.13–2.98)	2.44	0.01
6	0.82	2.26 (1.30–3.95)	2.89	0.004
Cows				
Age Class				
1	1.34	3.84 (1.52–9.70)	2.84	0.004
3	0.41	1.50 (0.59–3.82)	0.85	0.39
Herd				
PVB	–0.93	0.39 (0.16–0.97)	–2.02	0.04
SF	–0.59	0.55 (0.24–1.27)	–1.40	0.16
SJ7	–1.32	0.27 (0.09–0.83)	–2.28	0.02
SC	0.52	1.69 (0.81–3.53)	1.39	0.16

The probability of mortality from harvest was higher over the entire study period for both male and female elk than from all other documented causes of mortality; annual probabilities of mortality from other causes were generally nominal (Table 2.5). There was no statistically significant difference in mortality from legal harvest between sexes or age classes (Table 2.5a,b,c; Figure 2.2a,b). Point estimates for mortality from harvest varied among female herd locations, but 95% confidence intervals overlapped among estimates for all herd locations (Table 2.5d; Figure 2.2c).

Table 2.5. Estimated cause-specific mortality probabilities. Probabilities (95% CI) are from nonparametric cumulative incidence functions, based on results from the top sex-specific stratified Cox proportional hazards models (A.) by year, (B.) by male age class.

A.

Cause	Bulls			Cows		
	2011	2012	2013	2013	2014	2015
Legal harvest	0.18 (0.08–0.30)	0.44 (0.33–0.54)	0.29 (0.19–0.39)	0.39 (0.24–0.54)	0.28 (0.18–0.38)	0.25 (0.14-0.38)
Disease	0.08 (0.03–0.18)	0.02 (0.01–0.07)	0.04 (0.01–0.99)	0.02 (0.01–0.11)	0.03 (0.01–0.09)	0.00 (0.00-0.00)
Wounding loss	0.00 (0.00–0.00)	0.04 (0.02–0.10)	0.07 (0.03–0.15)	0.02 (0.01–0.11)	0.03 (0.01–0.09)	0.02 (0.01–0.09)
Other	0.00 (0.00–0.00)	0.07 (0.03–0.14)	0.07 (0.02–0.14)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)

B.

Cause	Age Class				
	2	3	4	5	6
Legal harvest	0.22 (0.08–0.41)	0.17 (0.08–0.30)	0.32 (0.21–0.43)	0.47 (0.33–0.61)	0.40 (0.24–0.56)
Disease	0.00 (0.00–0.00)	0.04 (0.01–0.13)	0.00 (0.00–0.00)	0.08 (0.02–0.18)	0.09 (0.02–0.21)
Wounding loss	0.00 (0.00–0.00)	0.04 (0.01–0.13)	0.06 (0.02–0.14)	0.02 (0.01–0.10)	0.09 (0.02–0.21)
Other	0.00 (0.00–0.00)	0.04 (0.01–0.13)	0.05 (0.01–0.12)	0.06 (0.02–0.16)	0.09 (0.02–0.21)

Table 2.5. (continued) (C.) by female age class

C.

Cause	Age Class		
	1	2	3
Legal harvest	0.45 (0.24–0.65)	0.27 (0.19–0.35)	0.31 (0.10–0.56)
Disease	0.00 (0.00–0.00)	0.02 (0.01–0.06)	0.00 (0.00–0.00)
Wounding loss	0.05 (0.01–0.20)	0.02 (0.01–0.05)	0.08 (0.01–0.30)
Other	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)

Table 2.5. (continued) (D). by female elk herd location.

D.

Cause	Herd				
	ATV	PVB	SF	SJ7	SC
Legal harvest	0.35 (0.22–0.47)	0.22 (0.10–0.39)	0.33 (0.19–0.48)	0.21 (0.10–0.35)	0.60 (0.07–0.90)
Disease	0.04 (0.01–0.12)	0.03 (0.01–0.14)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)
Wounding loss	0.06 (0.01–0.14)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.03 (0.01–0.13)	0.00 (0.00–0.00)
Other	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)

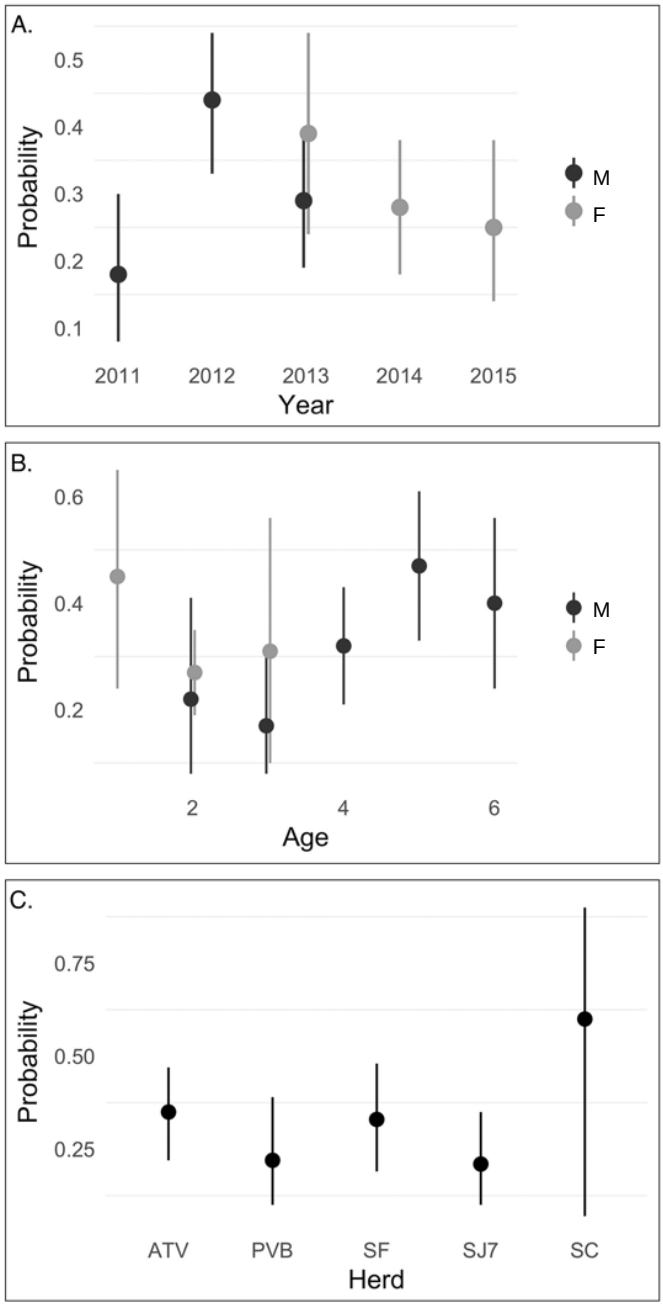

Figure 2.2. Estimated probabilities of mortality from legal harvest. (A.) By year, (B.) by age class for each sex, and (C.) individual female herds in southeastern Kentucky. Legal harvest was the most probable cause of mortality for both sexes, and probabilities of mortality from harvest did not vary between the sexes (B.) or among individual female herds (C.)

Discussion

Adult ungulate survival rates are high and fairly stable in non-hunted populations (Festa-Bianchet 2007). For example, reported survival rates of red deer (*Cervus elaphus*) and elk in non-hunted populations range from 0.9 to 1.0 for both males and females (Larkin et al. 2003b; Catchpole et al. 2004; Brodie et al. 2013). Survival of juvenile age classes (≤ 2 yoa) is highly variable and disproportionately influenced by population density, resource quality, and non-human predation compared to adult survival (Gaillard et al. 1998, 2000; Festa-Bianchet 2007). Nevertheless, legal hunter harvest is the primary cause of ungulate mortality globally and is a common tool used by managers to manipulate population demographics. To effectively manage populations to meet multiple objectives, an understanding of how different hunting strategies, particularly in relationship to land ownership types, influence survival is necessary.

Frequencies of harvest-related mortality in other states in eastern USA ranged from 10.0% in Pennsylvania (Banfield and Rosenberry 2015) to 58.0% in Michigan (Bender et al. 2005). This variation is largely due to state-specific harvest regulations, given the extirpation of most native large carnivores from the majority of the eastern USA (e.g., mountain lions [*Puma concolor*], gray wolves [*Canis lupus*], and red wolves [*Canis rufus*]). Survival rates of male elk in Kentucky are comparable to other hunted populations in North America but were generally towards the lower bound of the reported range. For example, annual survival rates of male elk range from 0.60 to 0.67 in Idaho, Alberta, and Michigan (Moran and Ozagoa 1973; Unsworth et al. 1993; Hegel et al. 2014), to greater than 0.80 in Washington (McCorquodale et al. 2011).

We found that survival probability of males was influenced by age class, with older age class males (≥ 5 yoa) having 2 to 2.5 times higher hazards of dying compared to younger age classes (Table 2.4). Although reports of survival probabilities by individual age class from other studies are limited, McCorquodale et al. (2011) reported no difference in survival between subadult (2-3 yoa) and adult (≥ 4 yoa) male elk in Washington, USA; whereas Biederbeck et al. (2001) reported a cumulative mortality rate of > 90.0% for males by the age of 4 in Oregon, USA. These differences likely stem from differences in harvest regulations between those two states. In Washington spike-only harvest was instated after several years of illegal killings (McCorquodale et al. 2011), whereas minimum point restrictions and any-male harvests were in effect in Oregon (Biederbeck et al. 2001). Although we did not include antler scores in our analysis, results from a survey of elk hunters in Kentucky suggested no intentional harvesting of males occurred based on antler size (KDFWR 2014). When presented with male elk (≥ 2 yoa) of varying antler and body size, an average of 79.7% (551/691) of surveyed hunters indicated a willingness to harvest any of the males presented (KDFWR 2014), suggesting that hunters in Kentucky do not selectively discriminate among males based on traditional trophy characteristics.

Similar to males, harvest was also the leading cause of female elk mortality in Kentucky (94.5% of deaths). The estimated average annual female survival rate of 0.67 (CI = 0.53 – 0.81) in this population is comparable to rates reported from other populations in North America, although it is also near the lower bound of reported ranges. Brodie et al. (2013) investigated 45 elk populations in the western USA and reported a mean female survival rate of 0.84, whereas Webb et al. (2011) reported a female survival rate of 0.80. Manipulation of adult female survival is the primary management tool used to influence

elk population dynamics, but a consistent trend of female survival as low as our estimate could negatively impact long-term population productivity and stability (Gaillard et al. 1998, 2000; Stalling et al. 2002). We also found that younger female elk had a nearly four-fold higher risk of mortality compared to adults (e.g., ≥ 2 yoa; Table 2.4). Recent research suggested that with increasing age, female elk may learn to avoid hunters and have a strong negative behavioral response to archery harvest techniques given the necessary proximity of archery hunters to individuals (Thurfjell et al. 2017). Although we did not have individual elk movement data in response to hunters, this learned behavior could explain the low survival rate of younger females.

Other causes of mortality such as vehicle collisions and disease were commonly reported in eastern USA elk populations but were infrequently reported in western USA elk populations (Keller et al. 2015). The probabilities of either sex dying from other causes were also low in Kentucky elk, including deaths from *P. tenuis*. *P. tenuis* infection was originally thought to be a serious concern for elk restoration efforts in the eastern USA (Larkin et al. 2001; Larkin et al. 2003a), but it resulted in only 12 mortalities among our sample of Kentucky elk (5.1%; Table 2.1). In contrast, a total of 24.0% of mortalities in the recently reintroduced elk population in Missouri, USA resulted from *P. tenuis* infection (Chitwood et al. 2018). This was more similar to what was reported in the Kentucky founder group, with 24.8% (36/145) of all mortalities being suspected from *P. tenuis* infection; although, only 5.5% (8/145) were confirmed (Larkin et al. 2003a). Density of white-tailed deer (*Odocoileaus virginianus*), a common host of *P. tenuis*, is considered an important factor in the prevalence of this parasite (Slomke et al. 1995). The presumed average density of deer in southeastern Kentucky is approximately 3.6 deer/km^2 (KDFWR,

unpublished data), whereas elk released into Missouri appear to inhabit an area with possibly greater deer density (Chitwood et al. 2018). Thus, *P. tenuis* may not be a concern in established elk populations, but it could still impede reintroduction efforts particularly in areas with moderate to high white-tailed deer densities.

During the first two years of our study, the elk restoration area was divided into six elk hunting zones. A total of 115 permits were available for males within the 2,670 km^2 zone that encompassed our study area. Point estimates of male survival during those first two years were 0.70 (95% CI = 0.58–0.80) and 0.43 (95% CI = 0.34–0.55), respectively, supporting a significant decline. Our study area included some of the few publicly accessible lands within the hunting zone, which KDFWR preemptively concluded caused hunters to congregate in the area and reduce male survival in 2012. Subsequently, KDFWR restructured hunting zones in 2013 into two 'at large' areas and three LEAs to attempt to reverse this trend (Figure 2.1). Our study area was entirely encompassed within one LEA, for which male harvest permits were also reduced by 55%. Although the point estimate of male survival in 2013 increased by ~ 32%, the 95% confidence interval for 2013 overlapped with both the 2011 and 2012 male survival confidence intervals, indicating the LEA was ineffective. We acknowledge that this could have been the result of other factors that we could not account for, such as sampling error or stochastic variation. In addition, male elk vulnerability to harvest is greater when the hunting season coincides with the rut (Hayes et al. 2002), as is the case in Kentucky. Nearly all harvest of male elk occurred within a 21 to 30-day period overlapping the peak of the rutting season. Therefore, continued monitoring of male survival and mortality is necessary to fully investigate the potential long-term efficacy of LEAs in Kentucky.

For the duration of female elk monitoring, the spatial extents of the LEAs did not change. The SF7, SF, PVB, and ATV female herds resided within the LEA (Figure 2.3), and variation in survival existed among these herds with members of the PVB and SF7 herds having lower hazards of dying (Table 2.4). Both the SF and SF7 herds primarily resided on privately owned lands, whereas the PVB and ATV herds mostly resided on public lands that differed in hunter accessibility (B.L.Slabach, unpublished data). PVB is a Wildlife Management Area that was closed to firearm hunting for elk, but it is bordered by land that was open to public hunting as part of a lease agreement. In contrast, the ATV site is a recreational area comprised of a matrix of public and private lands. At the time of our study, large portions of the ATV site were open to public hunting via both modern firearm and archery methods. Female mortality from firearms disproportionately affected the ATV herd with 46.0% (19/41) of all firearm mortalities occurring within this herd compared to 12.2% (2/41) in the PVB and SF7 herds (Table 2.1); although these raw frequencies were not significantly different ($\chi^2 = 3.34$, df = 2, $p = 0.18$). The proportion of archery mortalities was approximately equal among sites.

A total of 237 female elk permits were available during 2012 for the hunting zone that included our study area, resulting in a hunter density of 0.08 hunters per km^2 for female harvest. With the formation of the LEA in 2013, permits were reduced by 12.6%; however, the LEA encompassed 88.0% less land area than did the boundary of the original hunting zone (2,670 km^2 in 2012 vs. 312 km^2 in 2013). Therefore, hunter density for female harvest actually increased by 725.0%, given permits were nominally reduced with the formation of the LEA. Presumably, hunter density and pressure remained high for the 2013 and 2014 seasons. Thus, our results suggest that a limited-entry strategy that does not directly reduce

hunter density via a reduction in permits may allow hunters to continue congregating on more easily accessible public lands.

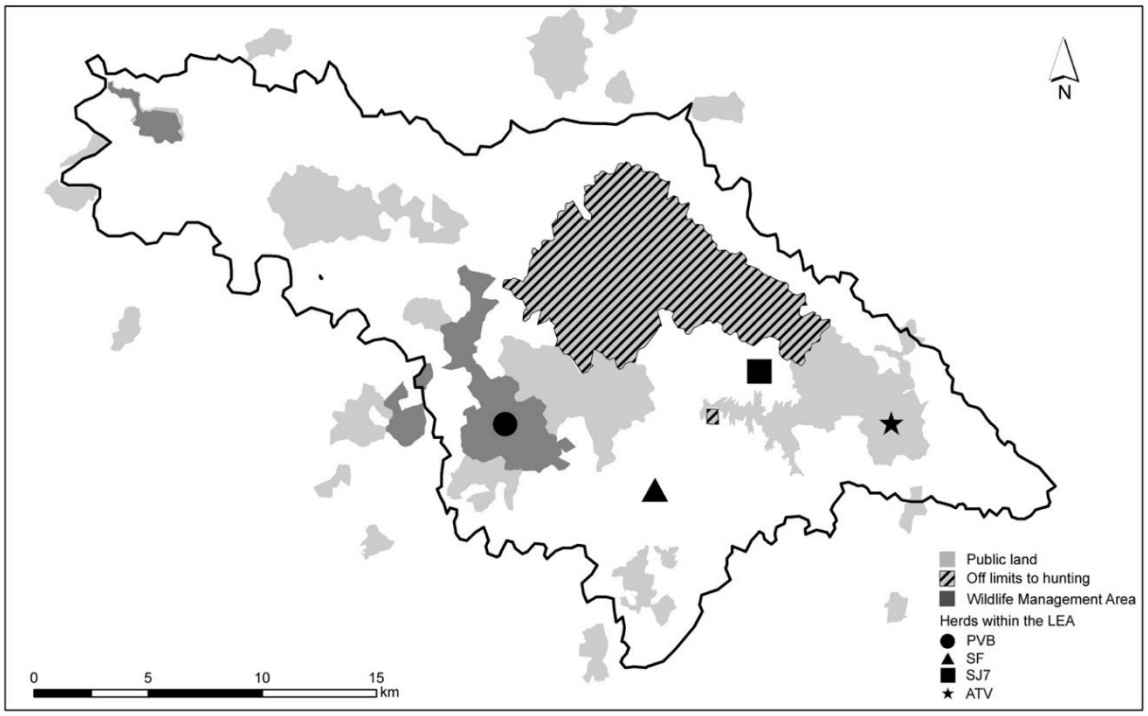

Figure 2.3. Differences in hunter access within the study area. These differences among sites resulted in varied hazards of death among female herds. The SF and SF7 herds mostly resided on private lands, whereas the PVB and ATV herds resided on mixed ownership sites. The PVB site is a Wildlife Management Area closed to firearm hunting for elk. Comparatively fewer lands that could serve as refugia from harvest were available for the ATV herd; thus, easier hunter access within the ATV site may have increased vulnerability of the ATV herd to harvest.

Management Implications

A majority of our study area was accessible by motorized vehicles. Both female and male elk select for habitats with lower human and road densities (Raedeke et al. 2002; Proffitt et al. 2013; Ranglack et al. 2017). In a mixed ownership landscape, this behavioral choice is especially important given the potential reduction of resident elk numbers via local overharvest or elk movement into refugia, reducing the number of elk available to hunters (Proffitt et al. 2013). In theory, a limited-entry strategy could reduce hunting pressure on older age class males and offset the high mortality rates that we observed in these age classes (Bender and Miller 1999). Because of hunter accessibility and limited availability of public lands, decreasing hunter density via further reductions in allotted annual permits would likely be a more successful management strategy for improving elk survival in Kentucky. Although a limited-entry strategy theoretically provides managers with improved control of hunting pressure and harvest within a given area, we suggest that this strategy should be informed by land ownership type, elk density, and sex-specific elk behavioral differences.

Acknowledgements

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CHAPTER THREE:

ELK (CERVUS CANADENSIS) SOCIAL STRUCTURE IN KENTUCKY: INVESTIGATING PATTERNS OF ASSOCIATION AND RELATEDNESS IN A HUMAN DOMINATED LANDSCAPE

Introduction

The type and patterning of individual relationships (social structure *sensu* Hinde 1976) provides pertinent insight into how ecological factors effect social behavior. The costs and benefits of sociality (e.g., grouping) vary with habitat heterogeneity, predator density and physiological state of individuals (Conradt and Roper 2003; Fortin et al. 2009). These factors also vary in time and across spatial scales, and temporally fluid association patterns – where individuals associate with a variety of others at different rates – are common among taxa (Krause and Ruxton 2002). For example, social carnivores such as hyenas and lions exhibit temporal variation in grouping as resources vary (Smith et al. 2008) and in response to territory and infant defense (Packer et al. 1990). Variation in flock size of parrots and macaws at clay licks are due to both interspecific competition for resources and predator avoidance (Brightsmith Villalobos 2011); and group size of many ungulates vary with changes in predation risk (Sundaresan et al. 2007; Valiex et al. 2009; Thaker et al. 2011).

Ungulates exhibit prime examples of highly fluid grouping patterns, forming both temporary and long-term associations (Altmann 1956; VanderWaal et al. 2014). Ungulate grouping is hypothesized to have evolved as an antipredator mechanism (Brashares et al. 2000; Geist 2006), with fluidity in group size and demographics hypothesized to be the result of individuals responding to changes in predation risk while balancing foraging costs (Gower et al. 2009). The cues to assess predation vary among species and in response to

different predator and habitat types (Thaker et al. 2011). Therefore, grouping patterns are highly variable across species and populations. For example, Valiex et al. (2009) found that long-term predation risk had a stronger effect on the distribution of browsing species versus grazing species. And individual studies of elk in the Greater Yellowstone ecosystem reported various, sometimes conflicting responses to wolf establishment (Gude et al. 2006; Creel and Winnie 2005; Gower et al. 2009; Profitt et al. 2009).

Human recreational use of landscapes results in behavioral changes that are synonymous to those of non-human predators. Recreational use increases vigilance (Ciuti et al. 2012b), alters space use (Profitt et al. 2013; Thurfjell et al 2017), and may induce similar physiological effects as predation risk by non-human predators (Creel and Christianson 2008; Creel et al. 2011). Ungulate populations experiencing constant predation pressure or frequent bouts of human disturbance trend towards smaller group sizes (Manor and Saltz 2003; Winnie and Creel 2007; Fortin et al. 2009). Smaller groups are less conspicuous; therefore, individuals may reside in smaller groups to reduce the risk of detection. Human predation (via hunting) has been shown to have a disproportionate effect on populations compared to non-human predators (Eberhardt et al. 2007; Milner et al. 2007). Managers use knowledge of behavioral changes and spatial distribution of individuals to inform management strategies and manipulate population demographics to meet management objectives. However, how human disturbance may directly or indirectly effect social structure or disrupt social processes isn't well known. For example, recent research has suggested that behaviors such as migration routes (Jesmer et al. 2018) and avoidance of hunters (Thurfjell et al. 2017) are culturally transmitted. And the spatial cohesion (e.g., tendency for individuals to remain in close proximity) of groups may be

influenced by the percentage of adults (Dolev et al. 2002). Therefore, in order to understand how human disturbance may affect group stability and social processes we need a better understanding of individual association patterns and the mechanisms that influence these patterns across social scales.

My objective was to investigate association patterns of female elk (*Cervus canadensis*) in Kentucky that inhabit a human dominated landscape. Elk form sex-specific groups outside of the breeding season and individual associations vary due to differences in antipredator strategies (Clutton-Brock 2009). I chose to focus on females given adult female survival disproportionally affects population dynamics (Gaillard et al. 1998, 2000). Like many cervid species, elk are matrilineal and female relatives often spatially overlap, and social relationships between kin or individuals of similar age are common (Clutton-Brock et al. 1982; Hirotani 1990; Clutton-Brock 2009). Female social units have been previously defined using home-range overlap and co-group membership (Altmann 1956; Millspaugh et al. 2004). Individual association preferences can be subtle or seem random in species that exhibit temporally, fluid associations. Using a network analytic approach, traditionally used in sociology (Whitehead 2008), provides tools to describe and investigate how individual associations result in the observed social structure (e.g., network). These methods have been applied to study the structure of vertebrate animal populations for the past decade (Whitehead 2008; Pinter-Wollman et al. 2014), although only a few studies have used these tools to describe ungulate social structure (Sundaresan et al. 2007; Carter et al. 2013; VanderWaal et al. 2014). First, I investigated differences in network metrics and group size between sites experiencing different types of human disturbance. I hypothesized that elk residing on landscapes with a higher frequency of human disturbance

would have smaller group sizes and more casual association patterns. Then, I investigated if association patterns were driven by phenotypic assortment or genetic relatedness at different social scales (e.g., subgroup, group, and site). I hypothesized that elk in this population would follow association patterns similar to other cervids where adult females associate more with relatives than non-relatives. Specifically, I hypothesized that genetic relatedness would be greater when comparing only elk within sites, similar to findings in red deer (*Cervus elaphus*; Nussey et al. 2005). I also hypothesized that association patterns at the group and subgroup level would be strongly influenced by age and genetic relatedness. Specifically, I hypothesized that individuals of similar age or relatives would have stronger associations.

Methods

Study area

The study area was approximately 300 km^2 and located in the Cumberland Plateau physiographic region in southeastern Kentucky (Figure 3.1). The climate and topography of the area have been previously described in chapter two. Surface mining operations have altered approximately 2300 km^2 of land within the region since the 1980s, resulting in a mosaic of native and exotic habitat that included open grasslands, second and third growth forests, active surface mines and mine reclamation areas (Larkin et al. 2001). The primary elk observation sites consisted of both active and reclaimed surface mined tracts, intermixed with primary and second growth forest where known elk herds frequented. The study area included several sites that differed in their public access and recreational use. These sites included a ~ 9km^2 tract of land dissected by gravel and dirt roads that served

as a Kentucky Department of Fish and Wildlife Resources (KDFWR) Wildlife Management Area (PVB); a 12 km^2 area that was privately owned and was both an active and reclaimed surface mine (SF); and a 32 km^2 public area comprised of meadow and forest with all-terrain vehicle access and horse trails (ATV). The ATV site was open year-round for public use including camping and several large outdoor concert and riding events throughout the year. All capture efforts were focused on herds residing at these three sites (Figure 3.2).

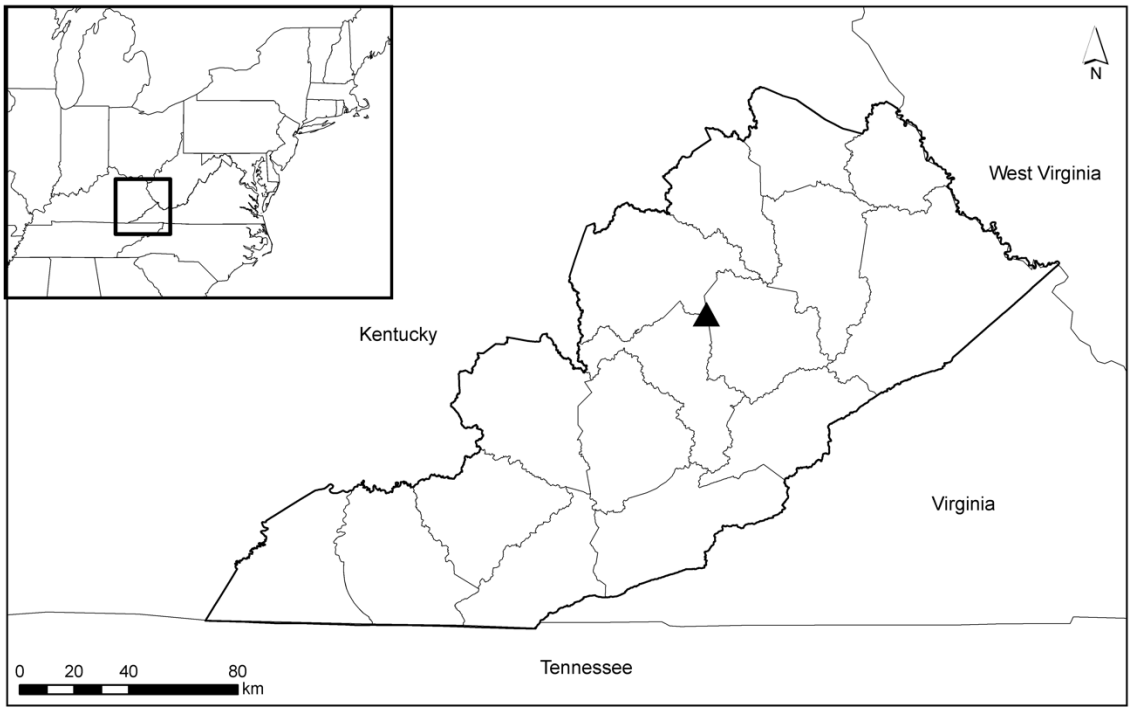

Figure 3.1. Map of the study location in southeastern Kentucky. This study took place within the 16-county elk restoration zone in the southeastern portion of the state (inlay).

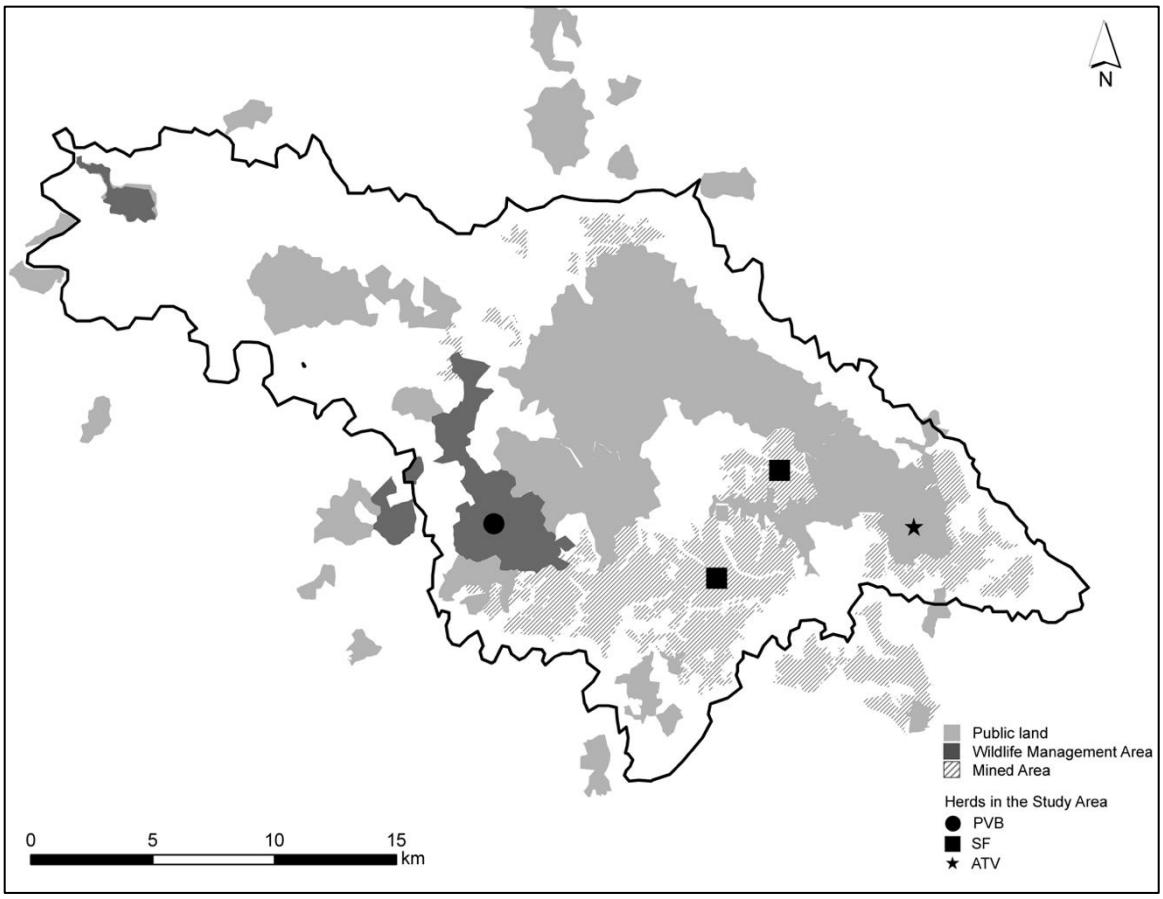

Figure 3.2. Differences in disturbance at elk capture sites. All capture efforts were focused on three sites in the 300 km^2 study area. These sites differed in their public access and recreational use. They included a wildlife management area (PVB), a recreational area with all-terrain vehicle access and horse trails (ATV), and an area that included both active and reclaimed surface mines (SF). Given the topography and accessibility of the landscape most observations occurred at the SF and ATV sites. Over the course of the study the ATV site remained public, whereas the SF site was a private site with limited recreational and hunting access.

Animal Capture and Identification

Female elk were captured from 1 February to 31 March 2013 and 2014. Capture efforts ceased by April 1 of each year to reduce the risk of injury to female elk and unborn calves. Capture methods are the same as previously described (see chapter 2). In short, elk were chemically immobilized using carfentanil citrate (Zoopharm, Windsor, Colorado, USA) at a dosage of 0.005–0.020 mg/kg of estimated body weight (Kreeger and Arnemo 2007), which was administered via a rifle-propelled dart or jabstick. When possible, calves or yearling elk were not chemically immobilized. Instead, a working chute with a cattle head gate (Tarter Gate Cattlemaster Series 3, Dunnville, KY, USA) was used to secure the animal. Each captured elk was outfitted with a very high frequency (VHF) radio collar (model: LMRT-4; Lotek, Newmarket, ON) that was individually marked using a colored banding pattern and numbered cattle ear tags to assist with individual identification. Total body length, hind foot length, and chest girth were measured for all captured elk, and 20 mL of blood was collected from the jugular vein for blood parameter analysis. Any signs of previous injuries or capture injuries were also noted. After elk were recovered they were monitored until they became ambulatory and were out of immediate danger of self-injury (~ 4.5 min on average). All capture and immobilization procedures were approved by a University of Kentucky International Animal Care and Use Committee (protocol #2010-0726).

Microsatellite Analysis

Tissue and hair samples collected from all marked elk (N_{all} = 94) were sent to Wildlife Genetics International (WGI; Nelson, British Columbia, Canada) for DNA extraction and

amplification. DNA was extracted using Qiagen's DNeasy Blood and Tissue kits (Qiagen, Valencia, CA, USA) with DNA quality management outlined in Woods et al. (1999) and Paetkau (2003). All samples were analyzed for 16 microsatellite markers that have been used in other studies of elk populations (Muller et al. 2018), including game-farmed elk. To minimize genotyping error, technicians discarded samples that failed at > 7 markers on the first pass of amplification and any samples that received low confidence scores were re-analyzed. I used MICO-CHECKER v2.2.3 (Van Oosterhout et al. 2004) to test for null alleles, allelic dropout, and scoring errors. I used GENEPOP v4.2 (Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium and to quantify linkage disequilibrium, applying a Bonferroni correction for multiple comparisons; and Spagedi v1.5 (Hardy and Vekemans 2002) to estimate relatedness (R) (Queller and Goodnight 1989) between all elk pairs within and between sites. Individuals were considered as member of a site if > 90.0% of direct observations and indirect (telemetry locations) of the individual occurred at a particular site. Standard errors for mean pairwise R estimates at specific sites were estimated by jackknifing across loci and significance was estimated using a permutation test (10000 replications), which permutes individuals among each subpopulation (e.g., sites).

Behavioral Monitoring

The topography and lack of access to private land did not lend itself to the use of transects to monitor association patterns. Consequently, I used continuous and scan sampling rules (Martin and Bateson 2007) to quantify association patterns between female elk from April to August in 2013, 2014, and 2015. Observations were restricted to this time

window due to the onset of the rut and hunting season (September – January) and subsequent capture window (February – March). Because elk in Kentucky have been observed to exhibit peaks in activity around crepuscular hours and typically bed in cover during midday hours (Olsson et al. 2006), I restricted behavioral data collection to crepuscular hours (three hours post dawn and three hours prior to sunset). A randomized list of individuals was generated weekly to determine the order of site visitations. Sites were visited 2 – 3 times per week on average (range = 2 -6). Elk were located using ground telemetry prior to the start of the observation window. Once visible, elk were continuously monitored for 60 minutes or until they were no longer visible, whichever came first. A group was defined as more than one elk foraging or traveling together with no individual > 50 meters from one another (Clutton-Brock et al. 1982). Group size, demography, and approximate spatial distance between individuals was recorded every 10 minutes. A minimum distance of 100-200 meters from groups was maintained, and both binoculars (10 x 40 mm) and a spotting scope (20-60 x 80 mm) were used to observe animals without interference. If elk were not visible, they were monitored via radio telemetry for 60 minutes prior to moving locations. Observations were conducted outside the April – August time frame when possible. Group size was calculated by averaging the observed group size across all ten-minute time points for each day.

Quantifying Association Patterns

Many individuals were observed over consecutive years, therefore I quantified individual association patterns by combing all years of the study. I constructed three different data sets to address my objective: all elk (N = 50) observed a minimum of 20

times, only elk observed at the ATV site (N_{ATV} = 28) and only elk observed at the SF site (N_{SF} = 25), excluding individuals sighted less 15 times. I filtered individuals to only include those sighted a minimum number of times to minimize the potential for false null associations. I also removed all calves (< 1 yoa) from the analysis. Three different females were observed at both sites and were maintained in each site-specific data set. I constructed a pairwise association matrix by calculating the half-weight index between pairs of elk (HWI; Whitehead 2008). This index has been used to quantify association strength in ungulates (VanderWaal et al. 2014; Tong et al. 2015) and controls for variation in observation frequencies among individuals. It is defined as:

$$\mathrm{HWI} = \frac{\mathrm{x}}{(\mathrm{x} + y_{12}) + 0.5(y_1 + y_2)}$$

where x is the number of times two individuals were seen together, y_{12} is the number of times individual 1 and individual 2 were seen but not together, y_1 is the number of times individual 1 was seen not with individual 2, and y_2 as the number of times individual 2 was seen without individual 1. The HWI index ranges from zero (a pair of elk was never seen associated) to one (a pair of elk was always seen associated). To further minimize the potential for false null association, I filtered all pairwise association matrices to only include HWI indices that were greater than the observed mean HWI for that dataset.

To determine if association patterns were different from random, I generated a distribution of HWI indices by permuting group membership in the R package asnipe (Farine 2018; R Core Team 2018). The permutation randomizes group membership, while preserving group size and the number of times each individual elk was observed (Bejder et al. 1998; Whitehead 2008). It performs by switching a 1 (associated) and a 0 (not associated) across rows of the group membership data (Bejder et al. 1998). The sampling

period was one day and therefore, I constrained the permutation to within a day by randomizing the ten-minute group time points for that day. I conducted the randomization 10,000 times to evaluate if the observed association preferences were different from random. I calculated the HWI indices after each iteration and compared the coefficient of variation (CV) of the observed HWI indices to the randomly permuted data, considering a significantly higher CV of the observed data as an indication of nonrandom association patterns (Whitehead 2008). I used this same method to test for nonrandom association patterns within each site using only the site-specific HWI association matrices.

To describe the social structure of female elk I used the observed HWI matrices to calculate the following network metrics:

(1) Degree: the number of individuals an elk is associated with calculated as a binary 0 (not associated) or 1 (associated). HWI values greater than zero were considered as an association.

(2) Eigenvector centrality: a measure of how well an individual is connected. It is calculated using how many associates an individual has and how well connected those associates are. Individuals with high eigenvector values are either highly connected or they are connected with others who also are highly connected.

(3) Strength: the sum of HWIs that an individual has with others. Individuals with a higher strength have strong preference to associate with certain individuals or have many associates. Strength is considered analogous to the gregariousness of an individual (Whitehead 2008).

To assess if observed network metrics were significantly different from random, I compared the mean observed network metric to a distribution of random means of the same

metric. I did this by generating 1000 random networks using the randomization technique previously described and considered the metrics significantly different if the mean observed metric fell within the outer 5% of the distribution of random means.

I also investigated whether associations occurred primarily within sites or between individuals within the same age class using a coefficient of assortment (New*r*) in the R package 'assortnet' (Newman 2003; Farine 2014, 2016). This measure calculates the proportion of total edge weights, where an edge is defined as a nonzero HWI between a pair of elk that occurs within a particular category (e.g., site). A New*r* value of one indicates that associations occur within the same categories, whereas a value of negative one indicates associations occur within different categories. The standard error (SE) of New*r* was calculated using a jackknife method across each category (Farine 2014). Elk were classified into three different age classes: (1) juvenile (≤ 2 yoa), (2) adult (3 – 8 yoa), and old adult (9+ yoa; Eberhardt et al. 2007). If an individual changed age class over the course of the study they were assigned to the age class they were last in. For example, if individual A moved from age class 1 to age class 2, they were assigned age class 2 for all analyses. To investigate the correlation between HWI strength and pairwise relatedness at different social scales, I used a Mantel test (Mantel 1967) with 1000 permutations implemented in the R package 'ape' (Paradis et al. 2004). I first investigated the relationship between pairwise relatedness and HWI strength for all elk and then only for elk within sites. I then filtered the site-specific data sets to only include those elk pairs with HWI value greater than 0.40, and considered groups (> 2 individuals) revealed through this filtering process as subgroups. I compared HWI strength to pairwise relatedness in subgroups, again using a Mantel test as previously described.

Results

Association Patterns between Females

I collected records of 951 elk groups with marked individuals over 2200 hours of observation effort. Most observations occurred at two sites (ATV and SF; Figure 3.2), given the topography of the landscape was more amenable to observation without disturbing groups. A total of 50 (N_{ATV} = 26; N_{SF} = 24) females were observed greater than 20 times (mean = 65, range = 1 - 189). Observed group sizes ranged from 2 – 53 (mean = 13.41, SE = 0.35, median = 11). There was no significant difference in mean daily group size between sites (mean SF = 14.6, mean ATV = 13.3, t = -1.79, p = 0.07). The percentage of marked individuals in the observed groups consisted of 4.0 – 100.0% at any point in time. The CV of HWI indices for all observed elk was significantly higher than random (CV = 1.62, random CV = 1.38, P = 0.002) indicating preference in companionships within this population (Figure 3.3). A similar trend was found at each site (ATV CV = 1.01, random CV = 0.99, P = 0.004; SF CV = 0.97, random CV = 0.79, P < 0.001). HWI values ranged from 0.02 - 0.62 (mean = 0.10, mean nonzero = 0.31, SE = 0.02). Mean nonzero HWI significantly differed between sites when all elk were considered (mean ATV = 0.32, mean SF = 0.38, t = 2.67, p = 0.034, 1000 permutations). There was a strong positive assortment by site (New*r* = 0.91, SE = 0.01, P < 0.05), but I found no assortment by age class (New*r* = -0.0002, SE = 0.27, P < 0.05). Mean nonzero HWI by age class was 0.31, 0.31 and 0.42, for juveniles, adult and old adults respectively. All network metrics tested differed significantly from random networks for all elk and for each site-specific data set (Table 3.1). Two different component groups occurred at the SF site compared to only one at the ATV site (Figure 3.3).

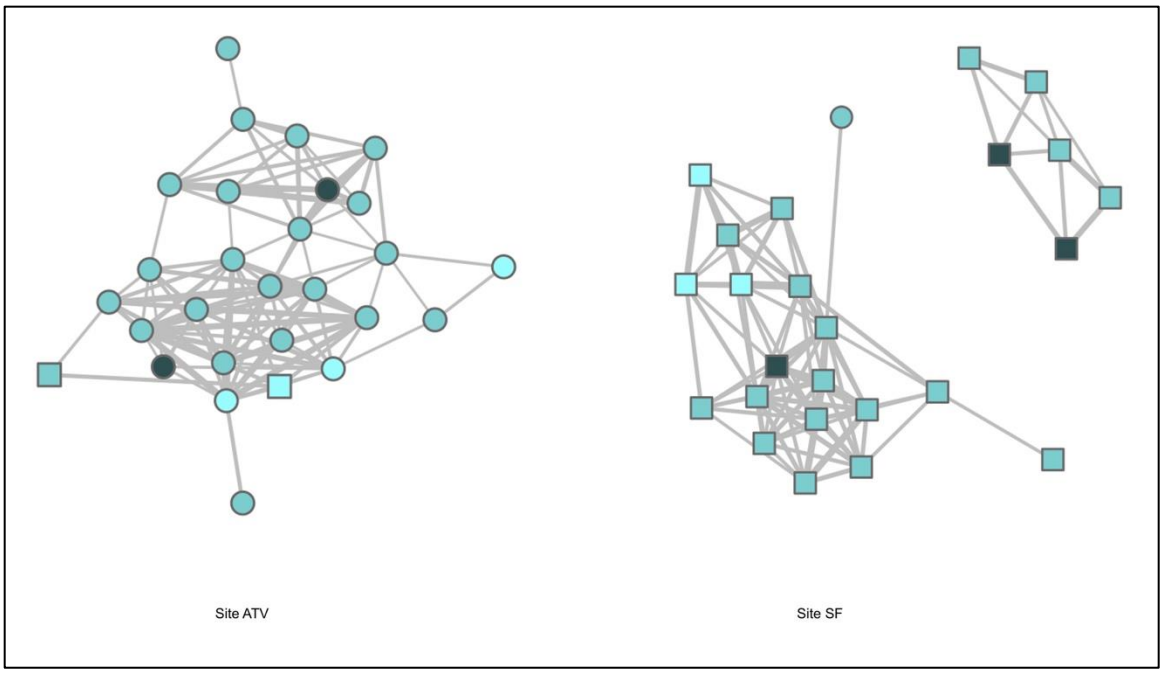

Figure 3.3. Network representation of female elk associations. Social network of all elk observed at ATV (circles) and SF (squares). Each node (circle or square) represents one elk and relationships between elk are represented by edges (lines between nodes). Node color depicts age class (lightest = juveniles, darkest = old adults), whereas edge width depicts HWI (thicker lines = larger HWI). The distance between individuals has no value and is randomly generated for visual purposes only. No assortment by age class was found (New*r* = -0.0002, SE = 0.27, $P < 0.05$) overall. The CV of HWI differed from random for both sites (ATV CV = 1.01, random CV = 0.99, HWI, P = 0.004; SF CV = 0.97, random CV = 0.79, HWI, $P < 0.001$), indicating association preference within sites. The mean nonzero HWI was also significantly higher at the SF site (mean = 0.38, range = 0.26 – 0.69) compared to the ATV site (mean =0.32, range = 0.21 – 0.54) based on 1000 permutations.

Table 3.1. Average network metrics and comparable random metrics. Random metrics were generated from random networks calculated using the observed HWI matrices for each dataset. Median observed values are given in parentheses.

Network	HWI	Degree	Eigenvector	Strength
All (N = 50)	0.31	9.76 (11.00)	0.28 (0.09)	3.04 (3.24)
Random	0.25*	12.7*	0.37*	3.21*
ATV (N = 28)	0.32	7.92 (8.00)	0.39 (0.22)	2.51 (2.45)
Random	0.31**	8.39*	0.43**	2.6*
SF (N = 25)	0.38	7.36 (7.00)	0.47 (0.46)	2.81 (2.81)
Random	0.27*	13.26*	0.57*	3.65*

Significant differences from 10 000 random networks: *P < 0.05 **P< 0.005

Relatedness of Associates

There was no evidence of any loci deviating from Hardy-Weinberg equilibrium after Bonferroni correction ($\alpha < 0.003$). Non-random associations of alleles were detected in only 1 of 120 (0.9%) pairwise comparisons at site PVB following a Bonferroni correction ($\alpha < 0.0004$). All other sites demonstrated linkage equilibrium. I found no presence of null alleles, allelic dropout or scoring errors. I included all loci in the analyses because no consistent pattern of deviation from Hardy-Weinberg disequilibrium, linkage disequilibrium or null alleles were found at the same loci for all sites. Two individuals from the ATV site did not amplify and were excluded from the analysis. Therefore, relatedness was calculated for a total of 92 cow elk. Average relatedness was greater within sites (R_{within} = 0.013, SE = 0.012, P < 0.05) compared to between sites ($R_{between}$ = -0.021, SE = 0.003, N = 92, P < 0.05). Mean pairwise relatedness for all 244 elk pairs was -0.013 (SE = 0.012). I found no relationship between HWI and pairwise relatedness (Mantel test x1000: r = -0.06, N = 50, P = 0.25; Figure 3.4).

Pairwise relatedness for 111 pairs of elk at the ATV site and 92 pairs of elk at the SF site was low (ATV: mean = -0.03, SE = 0.02, N = 28; SF: mean = 0.01, SE = 0.01, N = 25). No significant relationship was found between HWI and pairwise relatedness at either site when all elk pairs were considered (Mantel test x 1000: ATV r = -0.12, P = 0.16; SF r = 0.02, P = 0.26). When only considering the 38 pairs of elk at the ATV site more related than by chance (R > 0; mean = 0.16, SE = 0.02) a significant negative relationship was found (Mantel test x 1000: r = -0.47, P = 0.001, N = 28); whereas a positive trend was found for the 44 pairs of elk at SF (mean = 0.15, SE = 0.01; Mantel x 1000: r = 0.55, P = 0.001, N = 25; Figure 3.5). Three subgroups of individuals were revealed at ATV (N_{A1} =

7, N_{A2}= 6, N_{A3} = 3), while two (N_{SF1} = 4, N_{SF2} = 14) were revealed at SF. I found a negative significant correlation between pairwise relatedness and HWI when only subgroups were considered (HWI > 0.4) at the ATV site (mean relatedness = -0.05, SE = 0.04; Mantel test x 1000: r = -0.06, P = 0.007); whereas HWI was positively correlated, although not significantly, with pairwise relatedness at the SF site (mean relatedness = 0.03, SE = 0.04; Mantel x 1000: r = 0.29, P = 0.07).

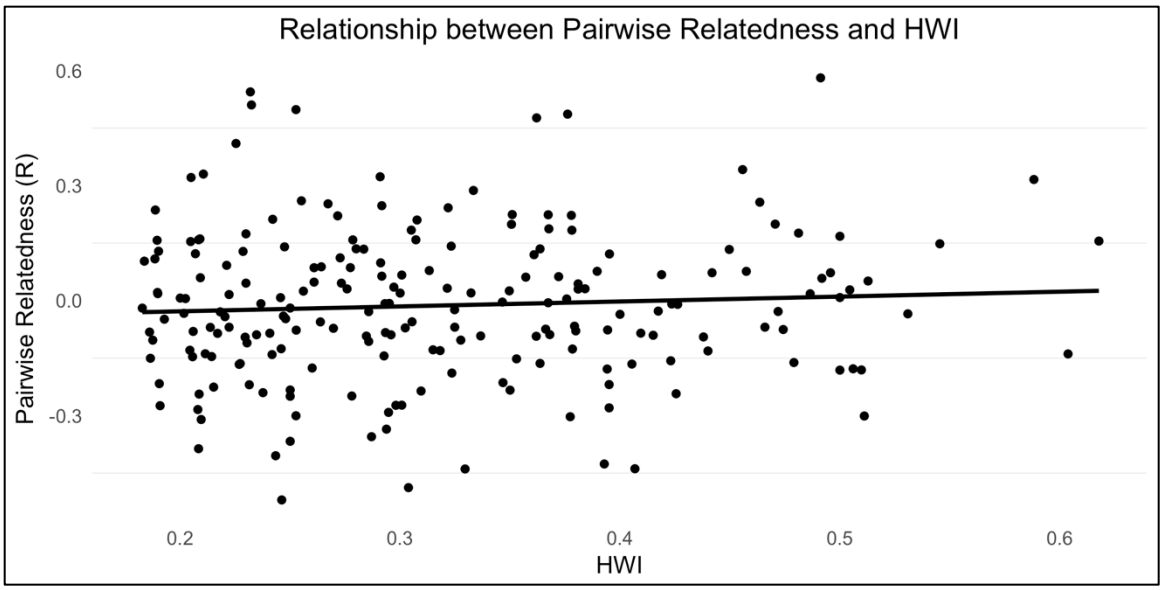

Figure 3.4. Relationship between pairwise relatedness and HWI for all elk. Mean pairwise relatedness for all elk pairs was low (mean = -0.013, SE = 0.012) and no relationship between HWI and pairwise relatedness was found (Mantel test x1000: r = -0.06, N = 50, P = 0.25). The trend was similar if only elk pairs more related than by chance (R > 0) were considered. Best fit trend line calculated in R (y = slope(x) + intercept).

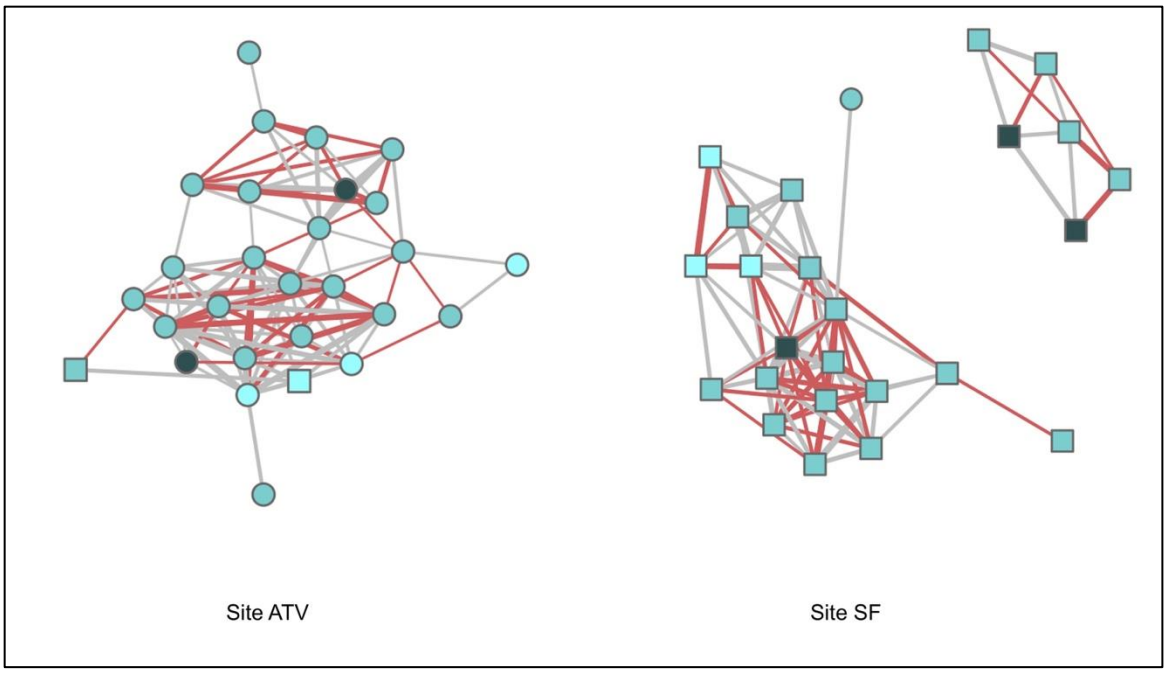

Figure 3.5. Network representation of female elk association patterns and relatedness. Pairwise relatedness between all observed elk at ATV (circles) and SF (squares). Node color depicts age class (lightest = juveniles, darkest = old adults). Edge width depicts HWI (thicker lines = larger HWI). Edges between related individuals (R > 0) are highlighted in red. The distance between individuals has no value and is randomly generated for visual purposes only. Relatedness within sites (R_{within} = 0.013, SE = 0.012, P < 0.05) was greater than between sites ($R_{between}$ = -0.021, SE = 0.003, P < 0.05) when all elk (N_{all} = 92) and all sites (N_{site} = 3) were considered. When only elk pairs more related than by chance (R > 0) were considered, a significant negative correlation between HWI and pairwise relatedness was found at ATV (Mantel test x 1000: r = -0.47, P = 0.001, N = 28), whereas a significant, positive relationship was found at SF (Mantel x 1000: r = 0.55, P = 0.001, N = 25).

Discussion

These results suggest that female elk in Kentucky have both casual and preferred associates. The observed mean overall HWI was low (0.06) but is comparable to other species that exhibit fluid associations such as giraffes (mean HWI = 0.07; Carter et al. 2013), Indo-Pacific dolphins (mean HWI = 0.02; Frere et al. 2010), and elephants (mean HWI = 0.06; Wittemyer et al. 2009). The mean number of associates (degree), mean eigenvector, and strength were significantly less than random (Table 3.1) suggesting that elk have strong preferences for particular individuals while casually associating with others. Grouping is an antipredator strategy (Hamilton 1971; Alexander 1974) and species that group in response to predation have strong social affinities by age class (Krause and Ruxton 2002; Clutton-Brock 2009). Contrary to my prediction, I found no evidence for assortment by age class (New*r* = -0.004, SE = 0.02, P > 0.05) or correlation between association strength and absolute difference of age of an elk pair (r^2 = 0.05, P = 0.18). Younger age class individuals have larger home ranges and are more prone to exploratory behavior (Clutton-Brock et al. 1982; Dolev at al. 2002; Haydon et al. 2008) and thus may be important links between subpopulations. There was no statistical difference in the eigenvector (one-way ANOVA: $F_{2,47}$ = 0.19, p = 0.83) or degree (one-way ANOVA: $F_{2,47}$ = 1.73, p = 0.19) between age classes in this population. Overall my results suggest that age is not a mechanism influencing association patterns.

Female cervids, such as elk and red deer (*Cervus elaphus*) tend to remain in their natal area and spatially overlap with relatives (Clutton-Brock et al. 1982; Nussey et al. 2005). The greater average relatedness within sites (R_{within} = 0.013, SE = 0.012, P < 0.05) and significant, positive assortment by site (New*r* = 0.91, SE = 0.01, P < 0.05) suggest that

individuals in this population follow that same trend. A total of 39.0% of all observed elk pairs were related and mean pairwise relatedness was negative (mean = -0.013, SE = 0.012). I found no correlation between pairwise relatedness and HWI strength between elk pairs (Mantel test x1000: r = 0.06, N = 50, P = 0.25; Figure 3.4). The lack of a relationship between HWI strength and pairwise relatedness could be the result of relatives reducing interspecific competition by not associating (Clutton-brock et al. 1982) or due to the small sample size. Approximately 37.0% (males and females) of the population within the study area was marked although direct observations were only possible on 5.3 – 6.8% of female elk.

Group sizes are smaller in populations under constant predation pressure (Mooring et al. 2004; Winnie and Creel 2007) or inhabiting areas with high human disturbance. Elk, bison (*Bison bison*), and mountain gazelle (*Gazella gazella*), all form smaller groups in the presence of hunters and other recreational landscape users (e.g., hikers, vehicles; Manor and Saltz 2003; Fortin et al. 2009; Ciuti et al. 2012b). Due to differences in disturbance type and frequency, I hypothesized that group size at the ATV site would be significantly less than the SF site. Yet I found no significant difference in mean daily group size at between the sites (mean SF = 14.6, mean ATV = 13.3, t = -1.79, p = 0.07). A majority of behavioral observations occurred during the summer months coinciding with the formation of nursery herds. Therefore, this lack of obvious difference could suggest an optimal group size during this time. Unfortunately, I did not have enough direct observations during the hunting season to compare changes in group size specifically in response to hunters on the landscape.

Association patterns within both sites follow similar trends, although two component groups occurred at SF versus only one at ATV (Figure 3.3). Each of the two groups at SF inhabited different areas of the SF site approximately 4 km apart and no intermixing occurred. The mean eigenvector and strength at both the ATV and SF sites were significantly less than those observed in random networks but both of metrics were also higher at the SF site (Table 3.1). This suggests that elk at SF are more highly interconnected. In comparison, the lower mean HWI and lower strength at ATV suggest while elk are interconnected, relationships are more casual compared to those at the SF site. Although relatedness was not correlated with association strength when considering all elk, relatedness was correlated to HWI within sites. A positive correlation between HWI and relatedness was found at both the group (mean = 0.15, SE = 0.01; Mantel x 1000: r = 0.55, P = 0.001) and subgroup level at SF (mean = 0.03, SE = 0.04; Mantel x 1000: r = 0.29, P = 0.07), although this relationship was only significant at the group level. This relationship did not hold at the ATV site where a significant negative relationship was found at the group (mean = 0.16, SE = 0.02; Mantel test x 1000: r = -0.47, P = 0.001, N = 28) and subgroup level (mean relatedness = -0.05, SE = 0.04; Mantel test x 1000: r = -0.06, P = 0.007). The frequency of first and second order relatives was equal at both sites and therefore could not explain this difference. Fluidity in ungulate grouping patterns are hypothesized to be the result of individuals responding to changes in predation risk while balancing foraging costs (Gower et al. 2009). Therefore, elk at ATV may have more casual relationships if they are changing groups or associates more frequently in response to human disturbance. The more casual relationships at ATV could also be an artifact of the marked population. Stable relationships between individuals may be present but occurring

between a marked and unmarked individual. The observed differences in association pattern and relatedness within sites may be best explained by differences in hunting mortality that occurred between the two sites.

Hunting is the primary cause of adult mortality of ungulates (Eberhardt et al. 2007). As a common game species that are highly regulated our understanding of the effect of harvest on elk social structure is minimal. Over the course of the study 40.0% and 71.0% of marked individuals at SF and ATV were removed due to hunter harvest (Figure 3.6). Elk at the SF site had a much lower hazard of dying due to harvest related deaths compared to those at the ATV site (Table 2.4). The casual association pattern characteristic of ungulates might suggest that social structure would remain fairly resilient against removals as long as a sufficient number of individuals reside in the area. Recent evidence suggests that both migration (Jesmer et al. 2018) and avoidance of hunters (Thurfjell et al. 2017) are culturally transmitted learned behaviors. Therefore, the removal of adult females could directly influence the transfer of information through populations influencing individual behavior and population level processes.

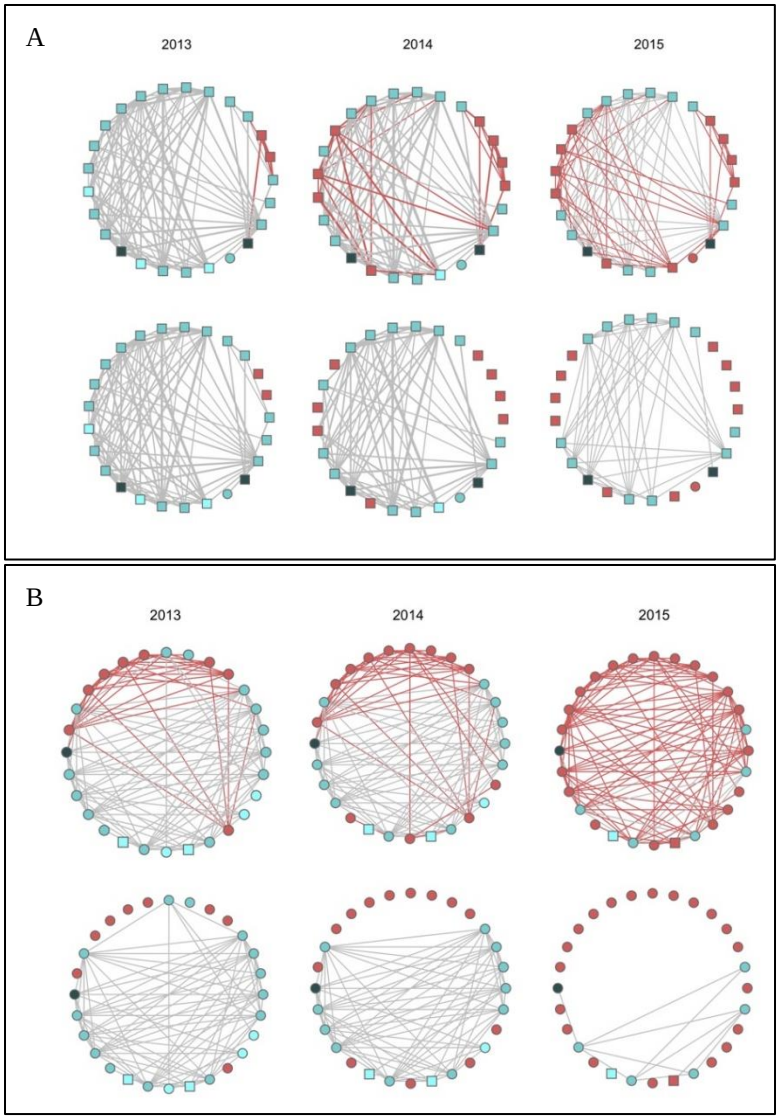

Figure 3.6. Visual representation of the effects of removal of elk due to hunter harvest. Over the course of the study a total of 40.0% of elk were removed from the SF site (panel A), whereas 71.0% of elk were removed at the ATV (panel B) site due to hunter harvest. The cumulative number of elk and edges removed are depicted in red (top row of each panel) and the density of edges within the network after removal is depicted in the bottom row. The extent of hunting that occurred at the ATV site could explain the observed negative relationship between relatedness and HWI at this site.

Factors that affect ungulate grouping are complex and operate at different temporal scales. Investigation of ungulate social structure has traditionally been limited to broad scale patterns and female elk have been reported to form social units, although results are conflicting (Altmann 1956; Knight 1970; Houston 1982; Millspaugh et al. 2004). Clutton-Brock et al. (1982) seminal work on red deer suggested subgroups were composed more of relatives and elk have been considered to follow this same pattern. More recent research has shown that pairwise relatedness did not influence the interaction duration of female elk (Vander Wal et al. 2012) suggesting this trend may not be universal. Therefore, group and subgroup structure could also be driven by behavioral or phenotypic mechanisms other than relatedness entirely. I only observed a total of 88 direct interactions between individuals over the course of the study, with a majority of these interactions occurring between a marked and unmarked individual. Elk, like many other prey species, have evolved phenotypic characteristics and cryptic forms of communication that curtail predator detection. And quantifying these effects in wild populations is logistically difficult. Considering the frequency of time individuals spend in close (< 6 meters) proximity to one another may provide a better description of elk social structure and the effects of disturbance. For instance, social cohesion or the propensity for individuals to remain in close proximity may be greater in subpopulations with a higher percentage of adult females (Dolev et al. 2002). Therefore, removal of females could decrease group and subgroup cohesion and would have implications for spatial movement patterns, information and disease transmission throughout the population. Future studies investigating cohesion and how association patterns change hourly or with age would

provide a better indication of the true dynamism of these populations and the potential mechanisms that influence these dynamics.

Overall, my results provide evidence that elk social structure is composed of both preferred associates and casual relationships. While the mechanism or mechanisms influencing the observed differences in association patterns are not completely clear, these results do provide evidence of how disturbance, particularly hunting may influence social structure of ungulates. Our understanding of how animals perceive human disturbance and the processes that effect grouping patterns in species that exhibit fluid social patterns have both became more evident in the past decade. And although elk are a highly sought after and regulated game species, our understanding of their social structure and the mechanisms influencing sociality is still lacking. A better understanding of individual association patterns across social scales is necessary in order to mitigate the potential effects of disturbance on population processes and viability.

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CHAPTER FOUR:

A MATTER OF TASTE? GEOPHAGIC BEHAVIOR OF FREE-RANGING UNGULATES ON A SURFACE MINE

Coauthors: J.T.Hast, C.D. Barton, and J.J.Cox

Introduction

Geophagic behavior, the intentional consumption of earth matter, is observed in a variety of organisms (Ayotte et al. 2006; Young et al. 2011; Slabach et al. 2015). Geophagy is considered adaptive in both human and non-human organisms where ingestion of soils or clay deposits can result in digestive benefits broadly attributed to nutrient supplementation, detoxification and remediation of osmotic imbalances (Mahaney and Krishnamani 2003; Ayotte et al. 2006; Young et al. 2011). Many herbivorous mammals seek sodium (Na) and calcium (Ca) from mineral licks particularly during times of high physiological demand such as pregnancy and lactation, or antler growth (Atwood and Weeks 2003; Wilson 2003; Slabach et al. 2015). Preference for soil type and seasonality of visitation to lick sites has been shown to vary between species, presumably due to species-specific nutrient deficiencies and/or site-specific variation in availability in nutrients (Ayotte et al. 2006).

Industrial landscape developments including gas well installation and mining operations have been shown to influence both species distribution and overall biodiversity of both plants and animals (Hebblewhite 2008; Hall et al. 2010; Bernhardt et al. 2012). Coal mining operations began in the eastern Kentucky Coalfields in the eighteenth century, with approximately 50% of coal production occurring in the eastern coalfields physiographic region (Nazzaro 2009). These operations dramatically alter both topography

and geochemical properties of soil, creating soils with nutrient concentrations that may emulate those found at natural lick sites. The chemical properties of mine soil depend heavily on the composition and weathering of mine spoil – all non-coal materials – that are used to create surface soil (Zipper et al. 2013). Un-weathered mine spoil oxidizes, increasing concentrations of sulfates, soluble salts and other trace elements, such as in the case of pyrite and calcite oxidation (Jambor et al. 2000; Barton et al. 2003). Increased concentrations of sulfates and heavy metals in these soils could be physiologically toxic to organisms if ingested (Plass 2000). However, un-weathered surface soil composed from mixed sandstone may also harbor high concentrations of beneficial nutrients such as Ca, Na, magnesium (Mg), and potassium (K) that could attract animals (Zipper et al. 2013).

In 2013, three lick sites were discovered on coal surface mines in southeastern Kentucky. Geophagic behavior was subsequently observed at each site by elk (*Cervus canadensis*), white-tailed deer (*Odocoileus virginianus*), and turkey (*Meleagris gallopavo*) predominantly during the summer months. Elk in this area are known to favor the open grasslands of reclaimed mines and spend ~ 95% of their time on or near (< 0.5 km) these disturbed areas (Olsson et al. 2006). Reclaimed mine lands increasingly serve as important foraging habitat for wild ungulates and livestock throughout the eastern United States. Although a great deal of research has been invested in understanding the effects of, and recovery from, mining activity on soil and water resources (Zipper et al. 2013; Sena et al. 2014), vegetation (Holl 2002; Sena et al. 2015), and wildlife recovery (Larkin et al. 2008; Price et al. 2015); to date, research addressing any potential indirect effect of mining and reclamation on large mammal populations is scarce.

My colleagues and I aimed to investigate the nutrient concentrations of soils found at lick sites compared to non-lick control soils found elsewhere on the mined landscape. Geophagic behavior occurs in all species of North American ungulates. Ayotte et al. (2006) and Kreulen (1985) suggested ruminants forage for Na, Ca, Mg, and phosphorus (P). In this study, I focused on Na, Ca, Mg, P, as well as sulfur (S), iron (Fe), and selenium (Se) given their common occurrence on mine sites. We hypothesized that lick sites would have higher concentrations of important dietary nutrients (Na, Ca, Mg, and P) supporting the hypothesis that geophagic behavior is a means of nutrient supplementation in this population. To our knowledge, this is the first study to consider nutrient acquisition by elk on a reclaimed mine site known to be high in toxic elements.

Materials and Methods

Study area and population

The study area was composed of two surface mines located in Knott County, Kentucky (37.35^{o} N, 82.95^{o} W), with a mixed-mesophytic hardwood forest characteristic of surrounding undisturbed habitat (Larkin et al. 2001). Surface mines comprised approximately 10.0% of total land disturbance within the county (Nazzaro 2009). These areas are comprised of a random mix of soil and rock strata spread and smoothed to stabilize the surface to meet mining reclamation requirements. Vegetative cover is then cultivated, creating a mixed mosaic of open grassland habitat and undisturbed forest (Plass 2000).

Elk inhabit a 300 km^2 range that includes both surface mined and adjacent undisturbed forests. This study includes the only three known licks within the area. All lick sites were

dry (*sensu* Ayotte et al. 2006) and were not located near a known spring or riverbed. Rain was observed to create standing puddles at all sites. Lick sites were located ~ 1- 6 km apart and varied in size. Geophagic behavior was observed regularly at one lick site, while female elk were either pregnant or lactating. Elk calves were also observed ingesting soils at this site. This lick was approximately 571.5 cm long and 203.2 to 304.8 cm at its most narrow and widest points. All other lick sites were much smaller averaging 100 - 125 cm in length.

Soil sampling and analysis

Soil samples were collected from lick sites (n = 3) and control (N = 16) sites in 2014. Lick sites were defined as sites where direct observation of geophagic behavior had occurred or there was evidence of pawing and/or scraping. Soil sampling was conducted per Mahaney and Krishnamani (2003). Non-lick control soils were sampled from areas that were used by elk (based on direct observation and radio-telemetry), had similar soil texture and coloration as licks and occurred in similar topography. Four soil cores from the upper 15 cm of the soil profile were collected from each individual control and lick sampling site. Two samples were collected from the periphery and two from the central area of each lick. Core samples from control sites were taken at an average of 30.5 cm (central) and 91.4 cm (periphery) from each other. Core samples from each individual sampling site were combined and dried for 48 hours. Samples were analyzed in duplicate for 14 different concentrations of nutrients including: Na, Ca, Mg, P, S, K, Se, Fe, manganese (Mn), aluminum (Al), arsenic (As), copper (Cu), lead (Pb), and zinc (Zn). Elemental analyses were performed using an inductively coupled plasma–optical emission spectrometer (Varian-Vista-Pro-CCD Simultaneous, Varian Inc., Palo Alto, CA) after digestion in HNO_3

(U.S. Environmental Protection Agency, 1996, method 200.2). Quality assurance–quality control protocols were followed for all analytical procedures as outlined in U.S. Environmental Protection Agency (1994) method 6020. Three replicate readings (mean = 50 secs) were used to improve detection capabilities. Yttrium chloride (1mg L^{-1}) was used as an internal standard (Webb et al. 2014).

Statistical analysis

A logistic regression analysis was used to assess how nutrient concentrations influence the probability of soil being ingested. Samples were combined based on function (lick vs. control) with lick sites serving as the dependent variable due to the pre-existing bias of elk to lick soils. Elemental concentrations tend to be highly correlated (White 1983) therefore linear relationships between nutrients were assessed via bi-variate scatterplots and Pearson correlation coefficients prior to further analyses. Standard model selection techniques were used and variables of interest included in the final model were Na, Ca, Mg, P, and S. Specific sample locales were not included in the final model because they did not improve overall model fit and were not predictive. Adjusted odds ratios for each variable are presented with 95% confidence intervals. All data were log transformed prior to analysis due to differences in elemental concentrations and all statistical analyses were conducted in R (R Core Team 2018).

Results

Concentrations (mg kg^{-1}) of nutrients of interest varied between lick and control sites. Overall, control sites had higher concentrations of nearly all nutrients with the exception

of Al, Mg, and Na (Table 4.1). Lick sites had lower mean concentrations of all variables of interest including Ca, S, P, and Mg; and lick sites had a mean concentration of Na three times greater than control sites. An overall association between soil ingestion and at least one regressor was found ($\beta = 32.71$, $p = 0.03$). A negative relationship between soil ingestion and the concentration of S ($\beta = -2.17$, $p = 0.02$) was the only significant result, suggesting that with increased concentrations of S in the soil there is a decreased probability of soil ingestion by nearly 90.0% (Table 4.2).

Table 4.1. Mean elemental concentrations (mg kg^{-1}) found at lick and control sites. A total of 3 lick and 16 control sites were sampled in 2014 for elemental analysis. All sampling took place on reclaimed mine sites. Control soils had higher concentrations of all elementals, with the exception of sodium (Na), manganese (Mn), and aluminum (Al).

	Control (mean + SD) n = 16	Lick (mean + SD) n = 3	Adjacent Forest*
Al	4545.18 (2606.68)	4609.31 (2084.63)	10257.42
As	5.85 (3.87)	3.77 (1.49)	
Ca	2926.19 (3173.87)	2066.98 (881.68)	287.02
Cu	24.46 (13.71)	14.96 (6.13)	
Fe	17993.78 (7669.42)	15046.43 (1286.55)	10514.64
K	1308.70 (708.05)	1261.12 (754.58)	1057.55
Mg	2202.89 (1019.72)	1374.33 (178.02)	572.85
Mn	330.22 (183.92)	371.18 (105.29)	66.71
Na	763.76 (1078.2)	3484.05 (4675.60)	67.01
P	248.11 (120.81)	148.84 (33.84)	
Pb	23.87 (14.88)	14.62 (3.06)	
S	1562.78 (1786.39)	331.29 (328.90)	
Se	1.08 (0.91)	0.76 (0.31)	
Zn	62.64 (25.37)	61.48 (35.11)	

*Soils collected at an undisturbed ridgetop landscape position in Robinson Forest which is adjacent to the mining complex. From Maharaj et al. 2007.

Table 4.2. Results of logistic regression analyzing how nutrient concentrations influenced the probability of a soil ingestion. After standard model selection, six elementals were included in the final analysis. An overall association between soil being ingested and at least one regressor was found ($\beta = 32.71$, $p = 0.03$). Sulfur was the only element that significantly influenced soil ingestion, with the probability of soil being ingested decreasing by nearly 90.0% with increased sulfur concentrations ($\beta = -2.17$, $p = 0.02$).

	β	OR	95% CI	p-value
(Intercept)	32.71	1.61E+14		0.03*
logNa	0.26	1.31	0.47, 3.30	0.57
logCa	-0.63	0.53	0.10, 1.99	0.40
logP	4.42	83.85	0.63, 17.14+E4	0.14
logMg	-5.63	0.004	0.08-E5, 1.09	0.09
logS	-2.17	0.11	0.01, 0.67	0.02*

Discussion

Concentrations for six (Ca, Fe, K, Mg, Mn and Na) of seven nutrients reported for an adjacent unmined forest (Maharaj et al. 2007) were elevated at both control and lick sites (Table 4.1). Al concentrations were lower on the mined sites as compared to the forest and is likely attributed to the lack of clay and excess of carbonates found in local mine soils (Agouridis et al. 2012; Sena et al. 2015). Jones and Hanson (1985) reported elevated levels of Ca (110 - 1,254 mg kg^{-1}), Na (150 - 510 mg kg^{-1}), and Mg (140 - 388 mg kg^{-1}) at five different undisturbed lick sites in Kentucky frequented by white-tailed deer. Sodium concentrations from our lick sites are comparable to those reported by Jones and Hanson (1985), yet our results show Ca and Mg concentrations much higher. Studies of other ungulate licks have reported similar ranges of various nutrient concentrations including those typically considered as most biologically important (Ca, Na, Mg, and P; Ayotte et al. 2006; Slabach et al. 2015). Ayotte et al. (2006) reported similar elevated concentrations in all nutrients from an unaltered (e.g., unmined) habitat in British Columbia.

We expected to find elevated concentrations of all nutrients at both lick and control sites due to the history of mining and reclamation on these sites. Both study areas have been extensively mined and active mining or reclamation work occurred up until 2013 in some areas (Kentucky Division of Mine Permits 2015). Unweathered mine spoil composed of mixed sandstone and shale is commonly used as a soil substitute during reclamation with this overburden high in Ca and Mg (Zipper et al. 2013; Daniels et al. 2016). MacMullum and Geist (1992) reported elevated average Na (6064 mg kg^{-1}), Ca (6620 mg kg^{-1}), and Mg (4220 mg kg^{-1}) concentrations from licks on exposed coal, overburden and

mine waste used by bighorn sheep (*Ovis canadensis*) and mule deer (*Odocoileus hemionus*) following this expected trend.

Due to the strong attraction ungulates have to Na and reported elevated levels of Na at lick sites the accepted hypothesis is that increased Na concentrations are the driving factor behind geophagic behavior in ungulates (Jones and Hanson 1985; Ayotte et al. 2006). Sodium was found at much greater concentrations at lick sites compared to control sites, suggesting that Na may be a driving factor of geophagy in this population (Table 4.1). The need for Na can increase 40% above baseline requirements during lactation and individuals can become Na deficient (Staaland et al. 1980). Yet Na concentration had no effect on the probability of soil ingestion in this study (β = 0.26, p = 0.57), although the observed lick use occurred predominantly post calving.

The presence of potentially beneficial nutrient concentrations alone, did not explain the preference for the observed lick sites in our findings. Ca, P, and Mg are necessary specifically during pregnancy and lactation (Cohen 1980). Phosphorus levels in ruminant milk remain constant despite variable P amounts in forage (Cohen 1980) making supplemental intake of P necessary. Phosphorus and Mg have been attributed to geophagic behavior in mountain goats (*Oreamnos americanus*; Slabach et al. 2015), white-tailed deer (Atwood and Weeks 2003), and bighorn sheep (Jokinen et al. 2014). We found no relationship between the probability of soil ingestion and Ca, P, or Mg concentrations at lick sites (Table 4.2).

Ungulates use licks for multiple reasons including to alleviate symptoms of gastrointestinal stress (Jones and Hanson 1985; Kreulen 1985; Ayotte et al. 2006, 2008) and to remedy osmotic balances in the digestive tract (Jones and Hanson 1985). Ungulate

visitation to lick sites commonly peak during the spring and summer coinciding with increased K concentration in forage (Jones and Hanson 1985). Increased K intake inhibits Na and Mg retention and can result in diuretic symptoms (Kreulen 1985; Ayotte et al. 2006). Loose fecal stools were not observed at any lick sites, nor during daily field monitoring. Direct observations of elk use of lick sites is limited given it was not the focus of the overall behavioral study. Observed visitation did occur predominantly during the summer, post calving and included female cow elk and calves (Slabach unpublished). This is consistent with the hypothesis that elk are seeking supplemental Na and Mg, in response to increase K in forage. Potassium concentrations at lick and control sites were approximately equal (Table 4.1). A complete vegetative and fecal analysis is necessary to test if lick visitation by elk is driven by gastrointestinal stress due to K concentration in forage.

Sulfur is a necessary element that is a component of hair and wool and it plays an important role in rumen metabolism (Church 1975). Jones and Hanson (1985) reported S concentrations from licks in Kentucky ranging between 22 – 840 mg kg^{-1}, comparable to the concentrations at our lick sites. Ayotte et al. (2006) reported sulfate concentrations between 400 – 4000 mg kg^{-1} at wet lick sites they investigated in undisturbed areas. We expected elevated S concentrations in all soils sampled due to the history of mining disturbance on these landscapes (Plass 2000). Yet concentrations at our control sites were nearly five times greater than those we found at the lick sites. Increased S concentrations reduced the probability of soil ingestion by 90.0% (95% CI: 0.01 – 0.67, β = -2.17, p = 0.02) suggesting that elk are preferentially choosing sites that are lower in potential toxic concentrations of S (Table 4.2).

Potential toxic effects of mining operations on terrestrial species have presumed to be limited to metal toxicity (Rodriguez-Estival et al. 2011; Ford & Beyer 2014). Sulfur toxicity is most commonly reported in domesticated animals as a result of elevated concentrations of S in feed or a primary water source. Consumption of excess S can result in decreased metabolic function, intoxication, and in severe cases, death (Gould 1998). Excess S intake can also create secondary deficiencies in other elements such as Cu and Se, a relationship reported in only one free-ranging ungulate population (Zhou et al. 2009).

Evidence has shown that ungulates can differentiate between essential nutrients and have an innate ability to taste harmful substances or avoid toxic levels of nutrients (Provenza 1996; Ceacero et al. 2010). Elk preferentially avoid food pellets high in Se (Pfister et al. 2015), and Ceacero et al. (2015) reported preferential avoidance of forage high in S in red deer (*Cervus elaphus*). Rarely do wild populations inhabit areas that have consistent, or permanently, elevated levels of both necessary and potentially harmful compounds. This study population is subjected to elevated concentrations of S across the landscape due to continued mining and reclamation. Water samples collected from this area had an average S concentration of 602.78 mg/L, compared to only 24.43 mg/L for Ca, 15.33 mg/L for Mg, and 11.79 mg/L for Na. This chronic exposure to high levels of S in water sources further supports that elk may be avoiding excess sulfur at lick sites.

Lick sites serve a variety of functions important to individual health and influence population distribution and habitat use (Festa-Bianchet 1988; Tobler et al. 2009; Slabach et al. 2015). Elk appear to be using lick sites to supplement necessary dietary elements, supporting our hypothesis and much previous work. Elevated nutrient concentrations were found at both lick and control sites, and in concentrations comparable to those reported in

other studies. Soil ingestion from anywhere on the landscape would result in nutrient supplementation. Therefore, geophagic behavior in this population led to compensation for nutritional deficiencies at licks that were lower in potentially toxic nutrients than surrounding areas.

Surface mining is increasing globally with increased demand for fossil fuels (UNEP 2011). Research on these impacts to ungulate populations have traditionally focused on habitat selection and population distribution (see Hebblewhite 2008 for a review). The mixed-mosaic landscape created by mining is suitable habitat for generalist herbivores and recolonization of mine sites by red deer (*C. elaphus*) has been reported (Muller et al. 2017). Reclaimed mines in the eastern United States are used as pastures for grazing livestock and for elk habitat. The open grasslands created by mining is considered necessary elk habitat; and further creation of grassland habitat through mining was considered beneficial to elk population growth in Kentucky (Schneider et al. 2006). However, our findings suggest that elk are modifying their foraging behavior to persist on these disturbed landscapes. With continued mining operations and the use of reclaimed mined areas as ungulate habitat, it is important to understand how ungulates may respond to chronic exposure to potentially toxic concentrations of nutrients.

In conclusion, our results suggest that in this population geophagic behavior is more strongly driven by the preference to avoid potentially toxic nutrients. Our results highlight an indirect consequence of mining on ungulate foraging behavior that has not previously been considered. Further research investigating the potential indirect effects of large scale mining operations on ungulate foraging behavior and distribution is warranted.

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CHAPTER FIVE:

A SURVEY OF TICK SPECIES IN A RECENTLY REINTRODUCED ELK POPULATION IN SOUTHEASTERN, KENTUCKY: POTENTIAL IMPLICATIONS FOR INTERSTATE TRANSLOCATION OF ZOONOTIC VECTORS

Reproduced from: Slabach, B.L, A. McKinney, J. Cunningham, J.T.Hast, and J.J.Cox. 2018. A survey of tick species in a recently reintroduced elk population in southeastern Kentucky: potential implications for interstate translocation of zoonotic vectors. JWD, 54(2): 366 – 370.

Introduction

Translocation and reintroduction are conservation management tactics that have increased in frequency within the past several decades (Larkin et al. 2003a; Seddon et al. 2005). Population supplementation and species reintroductions can be effective tools in meeting conservation goals but can result in unintended consequences such as translocation of vectors and more widespread distribution of pathogens (Le Gouar et al. 2012). Early reintroductions of elk (*Cervus canadensis*) to portions of their eastern range resulted in establishment of a few isolated populations (*e.g.* Pennsylvania, Michigan). Beginning in 1997, larger reintroductions occurred in central Appalachia (Kentucky, Tennessee, North Carolina, Virginia). Over a century after their extirpation in Kentucky, elk were released into southeastern Kentucky, USA, between 1997 and 2002 (Larkin et al. 2001). This population has since become a source population for translocation efforts of elk into Virginia, West Virginia and Wisconsin.

Historical information on tick distribution in Kentucky is variable (Bishopp and Trembley 1945; Kellogg et al. 1971), with more recent reports restricted to the central and western portions of the state (Taft et al. 2005; Pagac et al. 2014). *Dermacentor variabilis* and *Amblyomma americanum* have broad distributions in portions of Kentucky (Bishopp

and Trembley 1945; Kellogg et al. 1971; Alexy et al. 2004). *Dermacentor albipictus* distribution includes several counties; whereas *Ixodes scapularis* prevalence has increased in recent years across the state (Townsend 2016, 2017). *Amblyomma maculatum* has been reported in the western portion of the state (Paddock and Goddard 2015) but was not been reported in the eastern portion prior to the elk reintroduction. With continued translocation efforts planned improved understanding of parasite prevalence and distribution prior to translocation is necessary. My colleagues and I surveyed tick species currently residing on host elk in Kentucky to characterize relative abundance a decade post reintroduction. We aimed to contribute to current understanding of tick species distribution in Kentucky and assess the potential for translocation of tick vectors.

Methods

Ticks were collected from male elk from 2011 to 2013 and from female elk from 2012 – 2013 that were captured as part of an interstate translocation program and an intrastate radio-telemetry study. Elk capture occurred from January – April and June – July to avoid hunting seasons. No females were captured after April 1 to prevent injury to pregnant females; only data from male elk were available for June and July. All captures took place in southeastern Kentucky, USA, within a 16,802 km^2 elk restoration zone. Specific capture locations varied, yet a majority of all captures took place in Knott (37.3461° N, 82.9932° W), Breathitt (37.5360° N, 83.3362° W), and Perry (37.3161° N, 83.2078° W) counties (Figure 5.1). Ticks were collected from the face, ears and perianal areas of immobilized elk and stored in 95% ethanol. Samples were separated by species using reference specimens and identification keys. Mean intensity (mean number of ticks per infected host)

was defined per Rózsa et al. (2000). All statistical analyses were conducted using R version 3.2.1 (R Core Team 2018).

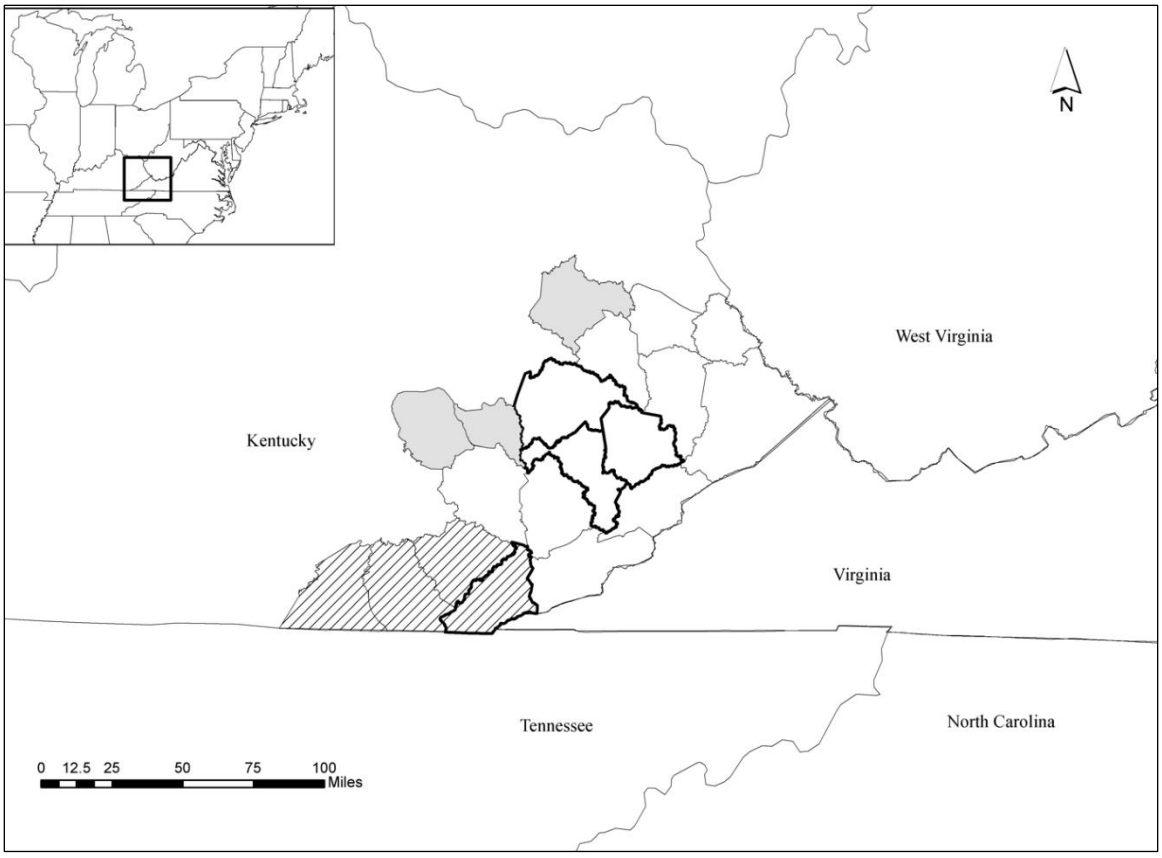

Figure 5.1. Map of study area and tick distribution in southeastern Kentucky. The elk restoration zone is comprised of a 16-county area. Only four counties within the elk restoration zone are included in known tick distribution maps (hatched lines), although species have been confirmed in counties surrounding the elk zone (light grey). Ticks were sampled from captured elk from 2011- 2013 in four counties Knott, Breathitt, Perry, and Bell (outlined in dark black), with a majority of captures occurring in Knott, Breathitt, and Perry counties which are not included in current tick distribution maps for Kentucky. Our results suggest that elk act as host to a variety of tick species in Kentucky and expand the current known distribution of tick species.

Results

A total of 1617 ticks representing five species were collected from 255 of 263 (97%) elk (F = 86; M = 177; Table 5.1). Total number of ticks collected by year during 2011, 2012, and 2013 were 388, 613 and 616, respectively. The most common species collected were *D. albipictus* (52.3%) and *D. variabilis* (42.1%). *A. maculatum, A. americanum,* and *I. scapularis* represented 3.3%, 1.4%, and 0.9%, respectively, of total specimens found. Bootstrap analysis (10000 iterations) revealed no significant difference between male and female elk in mean intensity of *D. variabilis* (mean = 2.6, p = 0.7, 95% CI: -2.6, 2.7) or *D. albipictus* (mean = 3.28, p = 0.27, 95% CI: -2.21, 2.07) tick when the 2012 and 2013 data were combined. Given female elk were not captured in 2011, no data were available to compare mean intensity between the sexes that year. Insufficient data were available to compare mean intensity of the additional three species collected.

Table 5.1 Number of elk sampled by year and county for tick analysis. Total number of ticks collected in each county are shown in parentheses. Female elk were not captured in 2011. A majority of captures occurred in Knott, Breathitt, and Perry counties.

	Elk Captured By County							
	Male				Female			
	Knott	Breathitt	Perry	Bell	Knott	Breathitt	Perry	Bell
2011	27 (149)	16 (118)	10 (63)	12 (58)	-	-	-	-
2012	32 (194)	26 (149)	12 (62)	12 (39)	4 (17)	3 (41)	23 (70)	10 (41)
2013	13 (163)	16 (157)	0	6 (31)	29 (178)	13 (78)	0	4 (9)

Table 5.2. Tick species collected by year. Overall 1617 ticks were collected from 255 of 263 (97.0%) of elk captured from 2011 – 2013. Five species were collected *Dermacentor variabilis*, *Amblyomma maculatum*, *Dermacentor albipictus*, *Ixodes scapularis*, and *Amblyomma americanum*. Individual proportion by species is shown. *D. albipictus* and *D. variabilis* represented the overwhelming majority of species found (94.4%).

Year	*D.albipictus*	*D.variabilis*	*A.americanum*	*I.scapularis*	*A.maculatum*	Ticks Collected	Elk Captured
2011	297	67	0	7	17	388	65
2012	335	218	19	4	37	613	117
2013	213	395	4	4	0	616	81
Total	845	680	23	15	54	1617	263
Proportion	52.3%	42.1%	1.4%	0.9%	3.3%		

Discussion

Our findings demonstrate that the recently established elk population acts as host to a variety of tick species and suggests a broader distribution of species than previously reported in Kentucky (Figure 5.1). We observed a higher infestation rate (97%, $N_{infected}$ = 255) compared to that previously reported on elk hosts (55%, $N_{infected2004}$ = 38 of 69 total elk collected) for the three-year period post reintroduction (Alexy et al. 2004). The frequencies of *D. albipictus*, *A. maculatum*, and *A. americanum* were approximately equal to those reported by Alexy et al. (2004). We observed a higher frequency of *D. variabilis* (42.1% vs. 1%).

All tick species occurred at low mean intensity. *I. scapularis* (mean = 0.05, SEM = 0.001), *A. americanum* (mean = 0.08, SEM = 0.001), and *A. maculatum* (mean = 0.21, SEM 0.004) occurred at the lowest mean intensities. The mean intensity of *D. variabilis* (2.6, SEM = 0.01) and *D. albipictus* (3.28, SEM = 0.01) was slightly lower than previously reported in this population (*D. albipictus* = 5.7), although the maximum number of ticks found on any elk infested with *D. albipictus* tick (max = 25) was comparable (max = 29; Alexy et al. 2004).

Sample collection was constrained by both elk hunting season (Sept. – Jan) and the inability to capture females during their third trimester of pregnancy (post April 1). *D. variabilis* can remain active in Kentucky throughout winter (Burg 2001) and our sampling times coincided with peak activity of *D. albipictus*. The overlap of our sampling with peak activities of both *D. albipictus* and D. variabilis, but not the other species, could explain the observed low occurrence of *A. americanum*, *I. scapularis*, and *A. maculatum*. Additional surveillance during peak activity of these species is warranted.

Ticks and tick-borne diseases are both an economic and human health concern and have long been recognized as an issue of concern in wildlife management (Daszak et al. 2000; Jongejan and Uilenburg 2005). Tick-borne zoonotic diseases have been increasing in recent decades (Jongejan and Uilenberg 2005; de la Fuente et al. 2008) and with increased prevalence has been linked to human-wildlife interactions such as increased host population (Barbour and Fish 1993). Treatment of animals prior to translocation can help avoid inadvertent spread of vectors. However many treatment regimes are commonly broad-spectrum antiparasitic that are not completely effective or require multiple doses (Wobeser 2002). This can increase the amount of animal handling and quarantine time necessary, both of which can have negative impacts on the animals and be detrimental to reintroduction efforts overall. Continued, non-targeted, treatment of animals post release is a potential strategy to control for introduced and native vector populations (Carroll et al. 2002). Continued surveillance of vector populations and maintenance of current distribution maps is necessary to prevent the inadvertent spread of vectors.

Reclaimed surface mined lands increasingly serve as important foraging habitat for livestock in eastern Kentucky causing extensive overlap of elk, white-tailed deer (*Odocoileaus virginianus*), and domesticated cattle. This overlap could explain the prevalence of *I. scapularis* we observed on elk, which have only previously been reported on white-tailed deer in Kentucky (Alexy et al. 2004). Overall this increased host density increases the potential for transmission of tick-borne zoonotic diseases that cause tularemia, babesiosis, bovine anaplasmosis, and lyme disease (Barbour and Fish 1993; de la Fuente et al. 2008). Future studies should include molecular investigation to determine the presence of any potential pathogens in these vector populations.

A. maculatum is found on >50 host species and is common throughout the southeastern US (Teele et al. 2010), although it had not been previously reported in the eastern portion of Kentucky prior to elk reintroduction. The introduction of elk to the southeastern portion of Kentucky could have augmented the distribution of *A. maculatum* in Kentucky. Only 4 *A. maculatum* specimens were reported post-elk introduction (3 specimens on elk, 1 specimen on a deer; Alexy et al. 2004). Although we only we recovered a small number (55 total) of specimens, the increased occurrence of *A. maculatum* on elk hosts does increase concern for wildlife and cattle infestation. With continued overlap of both wild and domestic host species we anticipate an increased occurrence of these vectors in the region.

Factors that influence the spread of vectors and tick-borne diseases are complex. Climatic factors, geographic shifts in hosts, and increased host density have all been linked to the spread of tick-borne diseases (Pfaffle et al. 2013). Understanding parasite abundance and distribution in source areas prior to reintroduction is necessary in order to understand the role of translocation in vector distribution. Up to date baseline distribution maps are important tools for managers to control infestations or spread of vectors that may lead to reintroduction failure. A priori monitoring is one of the primary strategies that can be employed to effectively avoid inadvertent spread of vectors and potential zoonotic pathogens.

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CHAPTER SIX:

CONCLUSION AND FUTURE DIRECTIONS

Quantifying association patterns between individuals is essential to understanding the social ecology of species. Identifying tiers of social structure, and the social and ecological factors that influence their composition, provides insight into the proximate and ultimate factors that affect sociality. The potential effect of human disturbance on animal population structure and dynamics has remained a concern for the past several decades (Ginsberg and Milner-Gulland 1994). We have documented how human presence alters habitat use (Proffitt et al. 2013), increases movement patterns and vigilance behaviors (Stankowich 2008; Ciuti et al. 2012b), and may preferentially select phenotypic traits (Ciuti et al. 2012a; Lone et al. 2015). In order to predict and mitigate the potential long-term effects of both consumptive (e.g., hunting) and non-consumptive human disturbance on animal populations, we need a better understanding of how these factors are compounded across social scales.

Temporally fluid association patterns are common among taxa (Krause and Ruxton 2002) and the ubiquity of these patterns suggests they are the rule and not the exception. Ungulate species exhibit one of the prime examples of fluid association patterns yet our understanding of associations between individuals is lacking. I aimed to identify the underlying structure and test hypotheses about the mechanisms that influence individual association and group formation, in an effort to better understand factors that influence sociality and better inform management of ungulate species. Early research documented stable subgroups of female elk (Altmann 1956) and while other studies also documented social units the scale at which they were defined makes it difficult for direct comparison (Jenkins and Starkey 1982; Millspaugh et al. 2004). My results suggest that females in this

population spatially overlap with relatives like other cervid species. Females have individuals that they prefer to associate with, but also maintain casual relationships with others. And these association preferences act as the foundation for groups within sites.

Management of populations relies on high quality field data to predictively model population dynamics, evaluate population viability, and inform management decisions. Most management strategies aim to reduce total population size via reduction of the adult female cohort, or increase availability of economically valuable males by increasing security habitat (Bender and Miller 1999) or reducing female density (Clutton-Brock et al. 2002). My colleagues and I demonstrated the importance of considering both sex-specific behaviors and hunter access in management decisions particularly in mixed use landscapes. Adult female survival is the primary factor influencing population growth and long-term population stability of ungulates (Gaillard et al. 1998, 2000). Although there is evidence to suggest stable female relationships underlie the social structure of ungulates, we rarely consider other consequences of hunting on females outside of the reduction of the total female cohort. By partnering traditional wildlife monitoring techniques with behavioral observations, my results highlight how human disturbance, particularly hunting, can directly influence social structure and result in more casual relationships between females. An increased proportion of casual relationships could directly impact socially transmitted information or disease spread and result in more spatially segregated populations, yet more investigation is needed.

Fluid patterns of associations are hypothesized to be the result of individuals balancing energetic costs and predation risk (Gower et al. 2009). Therefore, the observed patterns of more casual associations between females at the site experiencing a greater proportion of

human disturbance could simply be the result of individuals avoiding perceived risk in their environment over short temporal scales. What does fluid and short in this context truly mean? In order to answer that, I am investigating association patterns on finer time (e.g., < 10 min) and spatial scales. Given direct behavioral interactions between individuals are not very frequent (I only observed 88 direct interactions in total), defining an association using time spent in close proximity could provide a better indication of preferred associates and how these preferences change temporally. Studies using GPS collar technology to investigate home range or resource selection could add to this knowledge base by investigating overlap of individuals at various spatial scales.

Association patterns change with seasonal changes in resource distribution (Chapman and Chapman 2000; Wittemyer et al. 2005) and with predation risk (Sundaresan et al. 2007). Therefore, future studies investigating how association patterns change on defined short times scales (e.g., minutes or hours), in response to repeated non-lethal and lethal predation or with age are necessary to better understand and define this fluid phenomenon. Using dynamic models we can further investigate the resiliency of associations and group/networks to disturbance, how fluid associations may act to buffer populations on a local scale from extinction, and the threshold of fluidity.

Controlling and mitigating disease outbreak is difficult in wild ungulate populations and management plans for outbreaks are typically broad and not species specific. Empirically based network approaches such as separable temporal exponential random graph models have been used to understand disease transmission pathways through human populations, but to date have not been applied to animal populations. Incorporating temporal changes in association patterns on finer scales into models of disease transmission

will provide an important species-specific tool to mitigate outbreak. In addition, increased overlap between wildlife and livestock populations in the eastern United States could increase the prevalence of disease reservoirs and types of transmission pathways. Therefore a better understanding of disease dynamics on a community scale is necessary.

This research has also demonstrated potential indirect effects of coal surface mining on ungulate foraging behavior and the potential for translocating vectors with continued reintroduction efforts; highlighting the importance of future studies to consider both direct and indirect effects of large scale fragmentation, disturbance, and mitigation. As humans continue to alter and encroach on wild landscapes monitoring how animal behavior changes across spatial and social scales will help us to better mitigate and manage disturbance effects. It will also aid in our understanding of how animals are adapting, or not, to these changes in the landscape and thus, how human disturbance is exerting selective pressures. Overall, we (humans) are a part of an ecological community and understanding our effects on that community is a vital component to both species ecology and conservation.

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Education

2012	M.S.	Tufts University, Department of Biology
2009	B.A.	College of the Atlantic Human Ecology, focus in Animal Behavior, Ecology, & Conservation

Publications

*Undergraduate +Equal author contribution

B.L.Slabach and J.J.Krupa. 2018. Range expansion of the hispid cotton rat (*Sigmodon hispidus*) into reclaimed surface coal mines in eastern Kentucky. *In press at Southeastern Naturalist*.

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P.T.B.Starks and **B.L.Slabach**. 2012. The Scoop on Eating Dirt. *Scientific American Magazine*, 306(6): 30 – 32.

Professional Positions

2012 – 2018	Graduate Researcher and Teaching Assistant, University of Kentucky Dept. of Biology
2010 – 2012	Graduate Researcher and Teaching Assistant, Tufts University Dept. of Biology
2010	Field Coordinator, Alice Eno Research Station – Great Duck Island, ME

Honors & Awards

2018 Dean's Competitive Graduate Fellowship (Spring), College of Arts & Sciences, University of Kentucky
2016 Certificate of Outstanding Teaching, College of Arts & Sciences, University of Kentucky
2015 Best Student Poster, Tracy Farmer Institute for Sustainability and the Environment
2014 Best Student Poster, Society for Human Ecology